SAPONINS FROM INDIAN PLANTS

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The work described in this thesis has been carried out in the Department of Chemistry, Muslim University, Aligarh, under the guidance of Dr. I.P.Varshney, M.Sc.,Ph.D.(Alig.),Dr.ès Sc. (Paris), F.R.I.C.(London).

A C K N O W L E D G E M E N T

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C O N T E N T S

INTRODUCTION

I N T R O D U C T I O N

Saponin is one of the constituents found in plants and it can be either triterpenic or steroidal according to the nature of the sapogenin. Much attention has been devoted to this class of compounds due to the fact that steroidal genins are economically important being the precursors in the hormone syntheses. The triterpenic ones are academically important. Much work has been done on the saponins and sapogenins from various plants by Ruzicka, Jeger and collaborators in Switzerland; Barton Spring, Halsall etc. in England, Marker, Noller and Djerassi in America, and by Sannié, Lapin and Lederer in France, but practically very little work has been done on Indian plants. In the present thesis, the work on the saponin and sapogenin contents of a number of Indian plants belonging to the families Leguminosae and Cucurbitaceae has been described.

A. Family Leguminosae.

1. Sesbania aegyptica Pers: These seeds are a rich source of saponin and the genins obtained from these seeds have been investigated.

2. Sesbania aculeata,Pers: These seeds also contain a saponin which has been investigated.

3. Albizzia odoratissima,Benth from Maharashtra:

The seeds have been found to contain a mixture of two acid sapogenins.

4. Albizzia odoratissima,Benth from Uttar Pradesh:

These seeds contain a new triterpenic saponin Odoratissimin, yielding an acid genin.

5. Albizzia procera,Benth seeds from Maharashtra:

The seeds of Albizzia procera from Madhya Pradesh were earlier investigated, but the seeds from Maharashtra differed considerably and have been found to yield a new triterpenic saponin named Maharocin,

6. Albizzia lebbek,Benth flowers: As no work has been done on the flowers of any of the Albizzia species, these flowers have been studied and found to contain a triterpenic acid sapogenin and a mixture of flavonoids.

B. Cucurbitaceae.

7. Luffa aegyptica,Mill. These seeds have been found to contain a mixture of sapogenins which have been investigated.

The thesis contains the work on the saponins and sapogenins from the above plants, their isolation, characterisation and constitution. The genins have been obtained in micro-quantities and the work has been carried out utilising modern techniques.

THEORETICAL

G L Y C O S I D E S

The term glycoside denotes a combination of hydroxy compounds with various sugars. The non-sugar radicals may be simple or extremely complex aryl or alkyl compounds. The glycosides are hydrolysed by acids, alkalis or by specific hydrolytic enzymes furnishing saccharides or their oxidation products and aglycones. Oligo- and polysaccharides are the glycosidic condensation products of mono-saccharides, where the sugar units exert the dual function, one of the components behaving as a reducing sugar and the other one as an alcohol. But actually they are not classified under glycosides as they do not yield a non-sugar fragment on hydrolysis.

In 1837 a detailed study of amygdalin by Liebig and Wöhler formed the beginning of the study of the glycosides. Laurent[1] in 1852 placed such compounds which gave sugar on hydrolysis into a special group called "glucosamides". Berthelot[1] later termed them as "saccharides".

Finally the term glucoside was used because of the meagre knowledge, as no member was known at that time which

did not give glucose on hydrolysis. Later on when the presence of other sugars, in addition to glucose, was detected it was termed as glycoside.

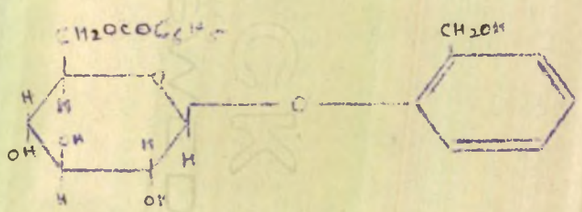
Classification of glycosides

As the principal interest lies in the aglycone portion, the classification of glycosides is based on the nature of this group. Following is a description of some of the glycosides.

1. Natural glycosides of alcohols and phenols:

(a) Phenolic glycosides.

Populin[2] (I) $C_{27}H_{22}O_8$ is a glucoside occuring in the bark, buds and leaves of certain species of Populus. On hydrolysis by taka-diastase it gives salicin and mono-benzoyl glucose. The attachment of benzoyl group on C-6 is shown by the acid hydrolysis of tetramethyl populin[2].

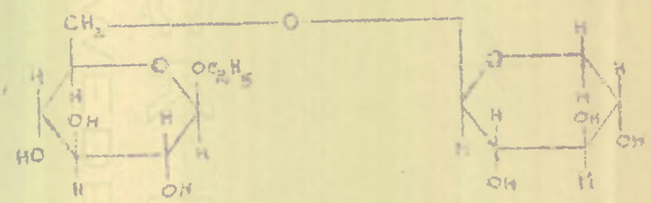


(I)

Populin

(b) Alcoholic glycosides.

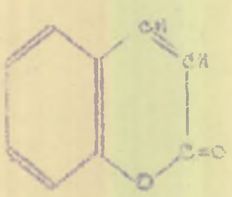
Gaultherioside[4] (II) m.p.185° isolated from fresh Gaultheria procumbens has got glucose and xylose as sugar moieties and ethyl alcohol as aglycone portion.



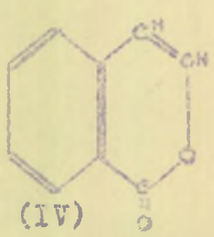
(II)

2. Coumarin glycosides

These glycosides are the derivatives of the lactone of O-hydroxy cinnamic acid. The structure of these glycosides is based upon coumarin (III) isocoumarin (IV) or their derivatives.

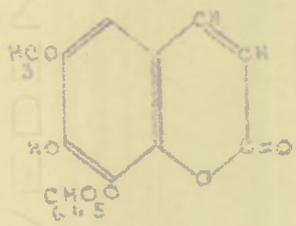


(III)
Coumarin



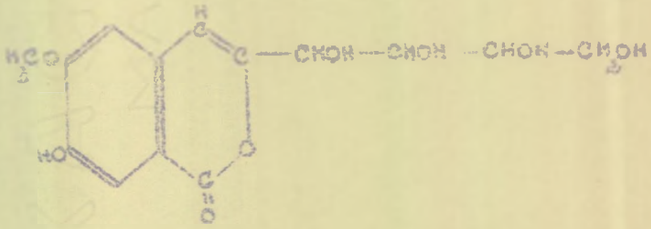
(IV)
Isocoumarin

(a) Fraxin[5], (V) $C_{16}H_{18}O_{10}$, m.p.205° is found in several species of Aesculus. On hydrolysis it yields 6-methoxy-7:8-dihydroxy coumarin $C_{10}H_8O_5$, m.p.227-228° and glucose. The glucose residue is attached to C-8.



(V)

(b) Bergenin (VI). $C_{14}H_{16}O_9 \cdot H_2O$. m.p.141°, anhydrous m.p.234° occurs as glycoside in Saxifraga crassifolia[6]. This is an isocoumarin derivative.

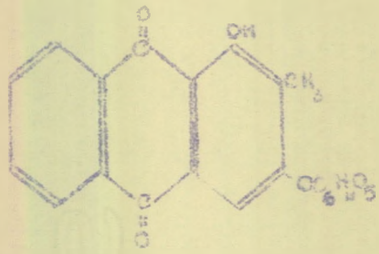


(VI)

3. Anthraquinone glycosides.

These glycosides are the anthraquinone derivatives

Rubiadin glucoside (VII) m.p. 270°, occurs with ruberythric acid in madder root. On hydrolysis it gives glucose and 1:3-dihydroxy 2-methyl anthraquinone.[7] According to Jones and Robertson[8] the attachment of the glucose is at position 3.

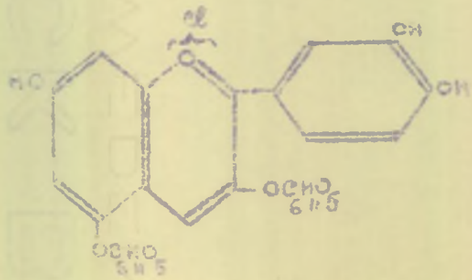


(VII)

4. Anthocyanin glycosides

They occur mostly in flowers as blue and red pigments. On acid hydrolysis they yield sugar and anthocyanidin chloride.

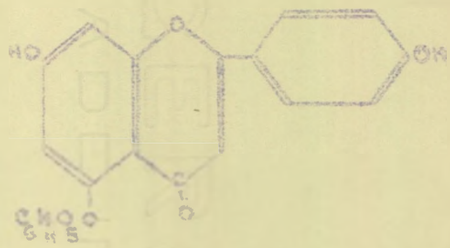
Cyanin chloride[9] (3,5-diglycosylcyanidin chloride) (VIII) $C_{23}H_{31}O_{16}Cl$ is the red or blue colouring matter of red roses and poppies and the blue corn flower.



(VIII)

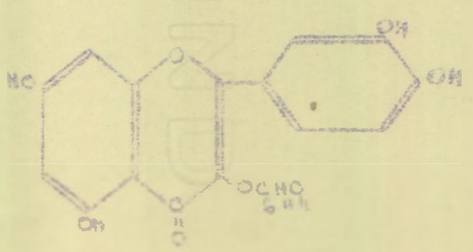
5. Anthoxanthin glycosides

(a) Apigenin-5-glucoside (IX) $C_{21}H_{20}O_{10}$ m.p. 295° is a flavone glycoside and has been isolated from the leaves of Amorpha fruticosa[10].



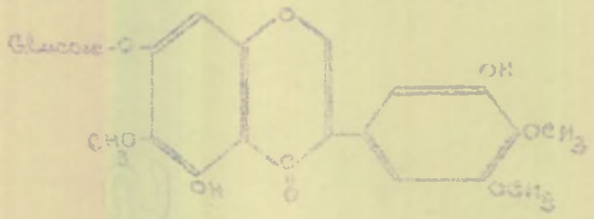
IX)

(b) Quercetin[8,11], (X) $C_{21}H_{20}O_{11}$, is found in the bark of Quercus tinctoria and has also been isolated from other sources. On hydrolysis with acid it yields quercetin and rhamnose. The sugar residue has been shown to be attached at position 3 of the aglycone.



X)

(c) Iridin[4] (XI) $C_{24}H_{26}O_{13}\ H_2O$, m.p. 208° is an isoflavone glycoside which occurs in Iris florentina. On hydrolysis it yields d-glucose and irigenin.

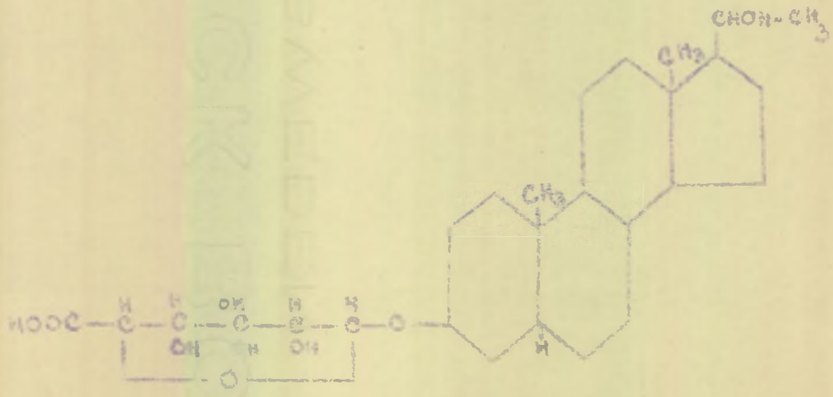


(XI)

6 - Hormonal glucuronides

A number of steroidal hormones are excreted by animals in urine in the glycosidic form in combination with glucuronic acid.

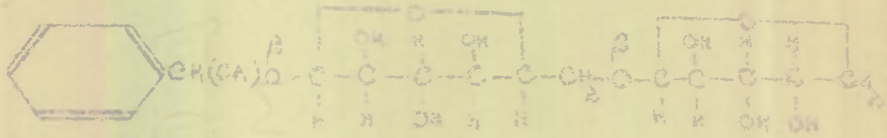
Pregnanediol glucuronide[12] (XII) $C_{27}H_{44}O_8$ is excreted in urine by women and dogs during their pregnancy period.



(XII)

7 - Cyanophoric glycosides:

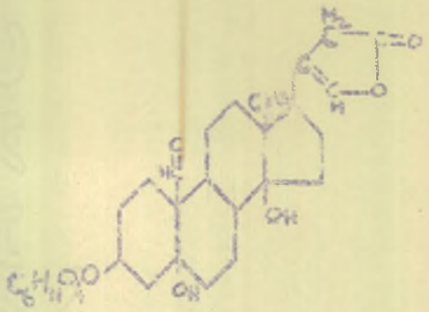
Vicianin[1] (XIII) $C_{19}H_{25}O_{10}N_7$, m.p.147-48° occurs in the seeds of wild vetch. Vicianase, an enzyme, hydrolyses it to Vicianose (a disaccharide) benzaldehyde and hydrogen cyanide. Acids and emulsin, hydrolyse vicianose to d-glucose and l-arabinose.



(XIII)

8. Cardiac glycosides:

These are the steroid glycosides with a heart stimulating action. Convallatoxin was first obtained by Karrer[14] from Convallaria majalis. Tschesche and Haupt[15] have established the molecular formula $C_{29}H_{42}O_{10}$. It has been assigned the following structure (XIV)[16], which on hydrolysis gives strophanthidin and rhamnose.

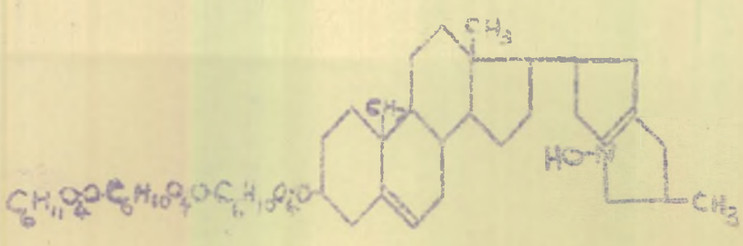


(XIV)

9. Solanum alkaloids

These are the nitrogen containing glycosides which have been isolated from various species of Solanum. In their physiological properties they are somewhat similar to saponins. The aglycones of these glycosides are alkaloids.

Solasonine (XV) $C_{45}H_{73}O_{16}N$, m.p. 275-76 has been isolated from Solanum sodomaeum[17]. On hydrolysis it yields solasodine as aglycone portion and rhamnose, galactose and glucose as sugar moieties. The glycoside[18] has been shown to have the following formula.



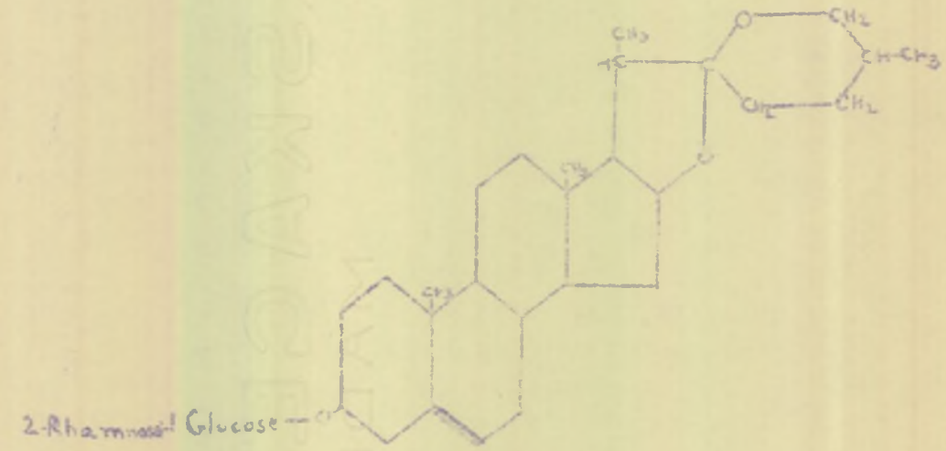
(XV)

10. Saponins: These are the glycosides separable into two classes according to the nature of the aglycone.

a) Steroidal saponins.

On dehydrogenation with selenium they yield Diels's hydrocarbon (3'-methyl cyclopentenophenanthrene) which indicates the presence of a steroid nucleus.

Dioscin[19] (XVI) $C_{45}H_{72}O_{16}$ $1\text{-}5H_2O$ has been isolated from Dioscorea tokoro. On hydrolysis with HCl it gives one mole of diosgenin one mole of D-glucose and two moles of L-rhamnose.

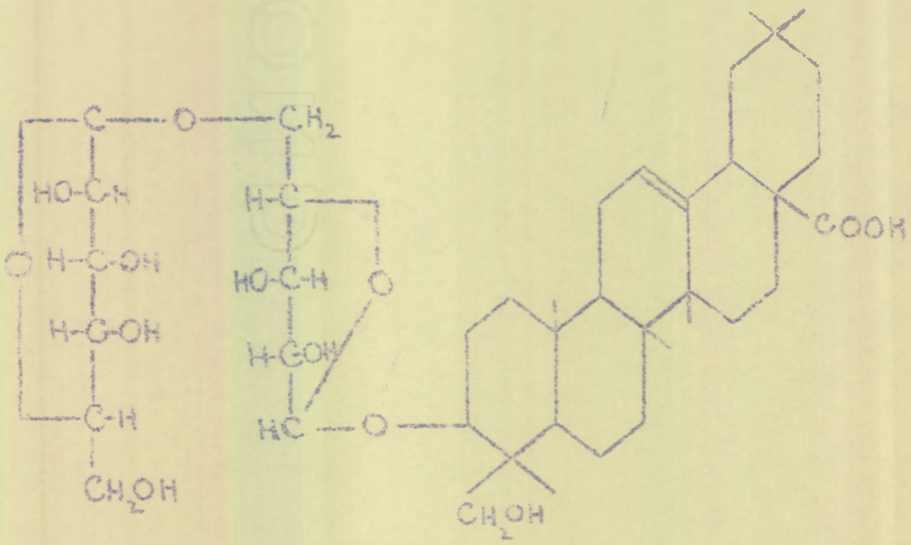


(XVI)

b)- <u>The triterpenic saponins:</u>

The dehydrogenation of triterpenic saponins furnishes a number of naphthalene derivatives.

Hederacoside[20] (XVII) m.p.257-60° is isolated from Hedera helix. On hydrolysis with sulphuric acid it gives hederagenin, glucose and arabinose.



(XVII)

SAPONINS

The plant glycosides which give copious foams on shaking with water are termed saponins. Some of the cardiac glycosides have also got the similar property, but due to their specific physiological action on the heart, they have been classified separately.

The detergent property of the saponins attracted attention at a very early stage. In the beginning the saponins were used for cleansing and washing of the body and clothes, but later they found use in washing of the delicate fabrics, which were damaged by the alkaline nature of the soaps and for this purpose they are still used in certain parts of India.

The word saponin is said to be derived from the word 'sapo' meaning soap[1]. There are divergent opinions about introducing the word "saponin" in literature. Richter[1] considers Grothas (1815) as the person who introduced the word. Wattiez and Sternon[21] credit it to Buchloz (1811), while in the opinion of Sannié[22] Gmelin (1819) was the man who used the word saponin for the first time.

The saponins on hydrolysis with acids or enzymes yield sugar, sugars or their oxidation products and aglycone or aglycones which are called sapogenins.

Properties

Physical: The property of foaming with water keeps the saponins into a separate class. It is due to the presence of saponins that soap-nut and "shakakai" are widely used for washing of silk fabrics and hair throughout India. The seeds of Randia locally known as "Mainphal" are commonly used by many villagers in Northern area of U.P. for the same purpose.

The saponins are rarely obtained in the crystalline form. They are generally isolated in the form of amorphous powder. Only a very few saponins are well defined crystalline compounds. Hederin[23] is one of the examples which is found in ivy (Hedera Helix) in crystalline form.

The saponins are soluble in water, dilute ethyl or methyl alcohols and to some extent, they are soluble in hot amyl, butyl and isopropyl alcohols also. They are usually in-soluble in acetone, chloroform, ether, petrol

and carbon tetrachlorides. The saponins are bitter in taste and cause sneezing[24]. They produce irritating effects on mucous membranes.[1,25]

The saponins dissolve in water forming colloidal solutions. They give stable foams with water, the stability of the foams is due to the low surface tension. The triterpenic saponins are used in the manufacture of accustic tile, photographic plates, films and papers, ceramics, foam fire extinguishers and tooth pastes etc.[25]

Chemical: The saponins are hydrolysed by mineral acids into aglycone called sapogenin and one or more molecules of sugar, sugars or their oxidation products. They can be hydrolysed by simply heating the saponin solutions with mineral acids or may need sometimes boiling and in few cases refluxing under high pressure.

The saponins form stable addition complexes with cholesterol and other 3 β -hydroxy steroids[25]. This property has been used for the purification of steroids as well as for the isolation of saponins. Digitonin, a saponin, is commonly used in the purification of steroids[26]. The digitonin complex of sterol, after dissolving in

pyridine, followed by precipitation with ether leaves free digitonin as insoluble precipitate and the sterol in solution. The pure sterol can now be obtained by the recovery of the solvent.[27]

The saponins are precipitated from their solution by lead acetate. Kobert[28], on this basis has divided them into three groups. In neutral solution the acid saponins are precipitated by neutral lead acetate, aqueous lead acetate precipitates a portion of the neutral saponins, while a third group remains in solution.

Kinzett[26] also mentions that the saponins can be divided into three classes, namely those precipitable by normal lead acetate, by basic lead acetate, and by basic lead acetate in presence of ammonia.

Physiological:

Dilute saponin solutions cause hæmolysis of red blood corpuscles and this phenomenon called hæmolytic effect has been observed upto the dilutions of 1:50,000 or even more[25]. The saponins are highly toxic to fishes, and for this reason the plants rich in saponins are used for stupefying and catching them, e.g. Balanites Aegyptica was used by the Arabs of the red Sea coast[30]. The saponin contents

are estimated by the method of "fish index". The "fish index" is the minimum concentration of saponin which will kill a fish (usually carp of 2-4 cm length) in one hour[30].

Another method suggested by Kobert[31] is the haemolytic index method. It is defined as the maximum dilution of saponin which still causes complete haemolysis of defibrinated blood at pH 7.48.

A few saponins like the saponin of the Chinese drug, Lien-Chiao[32] are known to be devoid of the haemolytic property.

The saponins have been noted to stimulate the penicillium growth[33]. The acceleration in the germination and growth of the wheat, gram and barley seeds by saponins has also been studied.

It has been reported by Varshney and Farooq[34] that the saponin of Albizzia lebbek Benth accelerates the wheat germination in 10^{-4} concentration, while at 10^{-2} concentration, it inhibits the germination. Also the seeds of gram and barley soaked for 24 hours in a 10^{-5} and 10^{-4} solution of the albizzia saponin[35] were

found to be accelarated in germination and initial growth of the plantlets. This stimulation is explained by the influence of the surface tension which permits the liquids to penetrate more rapidly, whereas higher concentrations inhibit the enzymes.

The fermentation of sugar by yeast is catalysed by saponins,[36] but the process is hindered or prevented in the presence of the salts [37]. It has also been found in some cases that certain concentrations of saponins adversely affect the process of fermentation.[38]

Therapeutic uses[23] of the saponins include expectorants, diuretics and antisyphilitics. In the latter case the efficacy has been found to be doubtful.

The saponin contents of a particular plant have been noted to vary with the season. The saponin content of the roots of Saponaria officinalis[39] was found to be highest during April and May, just before the appearance of flowers (7.74- 8.18%) and lowest during July and August, the flowering period (2.90%).

Isolation and purification of saponins

There is no general method for the isolation and purification of saponins. A method which works satisfactorily in one case may not be applicable in the other one. The isolation of a crude sample is not a difficult process, but it is the purification of the saponin which presents difficulties.

Generally, the saponin is extracted with dilute alcohols or water. The extract is concentrated to a small bulk and purified by different methods.

One of the method of purification consists in treating the water solution of the saponin with tannic acid[40] followed by neutralization with NaOH. The saponin tannate obtained as precipitate is suspended in boiling water and the tannic acid is precipitated by addition of zinc oxide, which is filtered off. The filtrate is evaporated to dryness from which the saponin is extracted by boiling methyl alcohol.

Kobert's lead acetate method[29] consists in adding an excess of neutral lead acetate to the saponin solution, which precipitates the lead complex of the saponin. It is

filtered and to the filtrate is added an excess of ammonia which precipitates any further amount of saponin left out in the solution. The two precipitates are suspended in boiling alcohol and decomposed by passing hydrogen sulphide. The method has not been found to be satisfactory as the products obtained are impure.

Extraction of the aqueous solution of the saponin containing 5% sodium chloride, with water saturated n-butyl alcohol, has been found satisfactory by Wall and collaborators[41]. The pH of the solution is adjusted between 4 and 5 by the addition of an appropriate quantity of HCl. The pure saponin goes into the water saturated butyl alcohol layer from which it is obtained by the recovery of the solvent under reduced pressure.

Marker and collaborators[42] have isolated some of the saponins by forming their cholesterol complex and its subsequent decomposition by boiling in pyridine. An excess of ether is added which precipitates the saponin, leaving cholesterol in solution.

A method used generally in the purification of the saponins consists in dissolving the saponin in small quantity of alcohol and precipitating it by addition to a large volume of ether or acetone. The process is repeated

several times, when a colourless saponin is obtained. The saponin may be purified further by passing through a bed of activated charcoal.

Another method of purification[43] consists in acetylation of the saponin with pyridine and acetic anhydride, and then deacetylating it by keeping the acetate in contact with a solution of NaOH of appropriate strength (0.4N) for about 24 hours or so. The alkali is neutralized by passing through a column of ion exchange resin. The saponin thus obtained is further purified by its precipitation with ether or acetone. There is no general method applicable to all the saponins. The method for the purification of individual saponin is devised in each case considering the nature of impurities present in the plant. As has already been stated earlier, one method which works well in one case may not be applicable in the other.

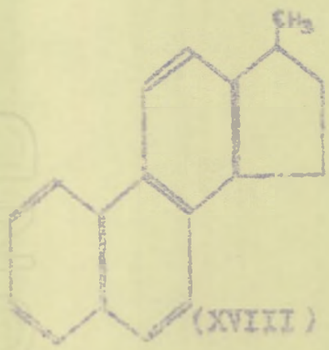
Constitution of Saponins

On the basis of the selenium dehydrogenation products, the saponins, have been divided into two separate classes.

(i) Triterpenic saponins

(ii) Steroidal saponins

The steroidal saponins and sapogenins on selenium dehydrogenation give Diel's hydrocarbon (3'-methyl-1,2-cyclopentanophenanthrene) (XVIII).



(XVIII)

(Diel's hydrocarbon or
3'-Methyl-1,2-cyclopentenophenanthrene)

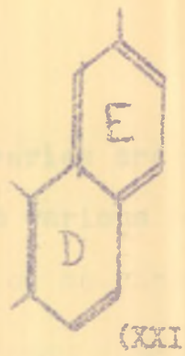
The triterpenic ones on similar treatment give a number of products which are largely naphthalene derivatives (XIX - XXVI)

(XIX)

1:2:3:4-tetramethyl benzene

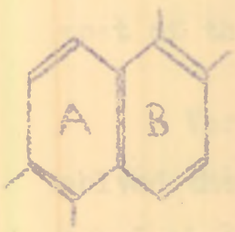
(XX)

2:7-dimethyl naphthalene



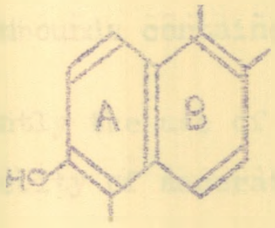
(XXI)

1:2:7-trimethyl naphthalene



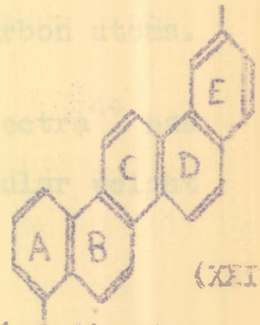
(XXII)

1:2:5:6-tetra methyl naphthalene



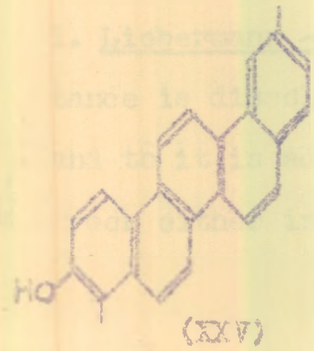
(XXIII)

6-hydroxy 1:2:5-trimethylnaphthalene.



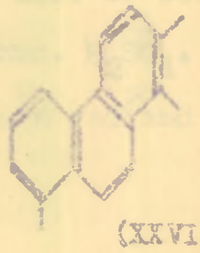
(XXIV)

1:8-dimethyl picene



(XXV)

1:8-dimethyl picene 2-ol



(XXVI)

1:2:8-trimethyl phenanthrene

Triterpenes

The compounds of triterpenic series are widely distributed in nature. They occur in various parts of the plants in free state, as esters, or as saponins.

The molecular formula determinations of the triterpenes have presented numerous difficulties. Results based on careful micro-combustions, determinations of the equivalents of acids by microtitrations, determinations of methoxyl value of esters and acetyl value of acetates showed in conjunction with isoprene rule that most of these compounds contained 30 carbon atoms.

Very recently the use of mass spectra[44] has solved the difficulty of accurate molecular weight determinations.

Colour reactions of triterpenes

1. Liebermann - Burchard reaction.[45] One mg. of the substance is dissolved by warming in 1cc. of acetic anhydride and to it is added one drop of conc. H_2SO_4. It becomes green either immediately or through red and blue shades.

2. Reaction of Salkowski.[45] To a mg. of the substance, as such or in chloroform, is added concentrated sulphuric acid (Sp. gr. 1.76). A yellow colour develops which gradually changes to dark red.

3. Reaction of Noller.[46] The substance (about 0.02 gm) and half cc of the reagent (0.01% pure stannic chloride in pure thionyl chloride) is taken in a test tube, which is corked and left aside. In the course of hours, a series of colour shades run through, but red is always there. The reaction is specific for triterpenes. Oxy-acids containing at least one OH group give a dark positive colouration.

4. Reaction of Kahlenberg.[47] To a solution of the substance in chloroform (0.2 cc; 1%) is added half cc. of the reagent (from a solution of 80 cc of $SbCl_5$ + 80 cc of $CHCl_3$). Dilution after five minutes with chloroform (10 cc) gives dark purple to blue colouration.

5. Reaction of tetra-nitromethane.[48] To a solution of the substance (few mg.) in chloroform are added one or two drops of tetranitromethane. The production of a yellow colour indicates the presence of a carbon carbon double bond. The test is not positive if the double bond is conjugated with a carbonyl group.

6. Chromatographic test.[49] A small quantity of the substance deposited on a filter paper when sprayed with a mixture of $SnCl_4$:AcOH:CCl_4 (6:50:50) and heated in an oven at 100°, gives a brown colouration. This test is specific for triterpenes, steroidal genins do not give this test.

7. Reaction of Sannie,[50,51,52] (Cinnamic aldehyde reaction). A deposit of a few mg. of the genin on a filter paper when sprayed with cinnamic aldehyde (1% ethanolic solution of cinnamic aldehyde) dried and resprayed with a mixture of acetic anhydride (12 cc) and sulphuric acid (1 cc) or alcohol, phosphoric acid and perchloric acid mixture (30:5:0.5) develops a yellow colour on heating, indicative of the presence of steroidal genins. The triterpenic genins do not respond to this colour reaction.

8. Whitby test.[53] To 2 cc of a $CHCl_3$ solution of the substance, when added 2 cc of a reagent prepared by mixing conc. H_2SO_4 and formalin (50:1) varying colours are formed. This test is given by steroidal genins.

9. Antimony trichloride reaction.[54] A piece of filter paper dipped in a solution of genin and in a chloroformic solution of $SbCl_3$ develops an orange colour when treated with a mixture of sulphuric acid and acetic anhydride.

This reaction, also indicates the presence of a double bond in 5-6 position in stercidal genins.

10. Reaction of Rosenthaler.[55] The addition of sulphuric acid to an alcoholic solution of genin containing vanillin hydrochloride develops a lilac colour.

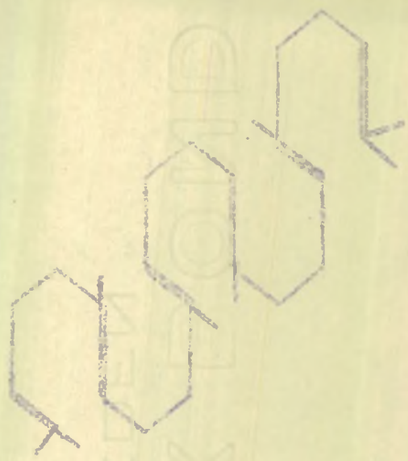
11. Reaction of Hirschsohn.[46] The substance on warming with trichlore acetic acid gives yellow to red colour.

The triterpenes can be divided into the following four groups.

(i) Acyclic-(Squalene) (XXVII)
(ii) Monocyclic- X
(iii) Bicyclic - X
(iv) Tricyclic-(Ambrein, (XXVIII)
(v) Tetracyclic-(Lanosterol (XLV), Euphol (XLIX).
(vi) Pentacyclic-(α-amyrin (XLIX), β-amyrin (L) and lupeol (LI).

(i) Acyclic (Squalene)

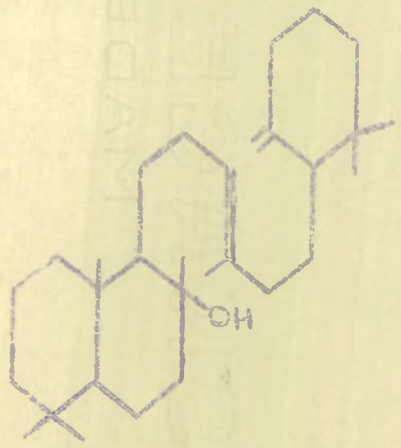
Squalene (XXVIII) has been found to occur widely distributed in nature. It has been isolated from varied sources, such as hair oil, fungi, human ear wax etc. Much of the work on the constitution of squalene has been carried out by Heilbron[56], Karrer[57] and their coworkers.



(XXVII)

(iv) Tricyclic triternenes (Ambrein).

Ambrein was first obtained from ambergris[58] in 1820 and has the following structure $C_{30}H_{52}O$ (XXVIII).



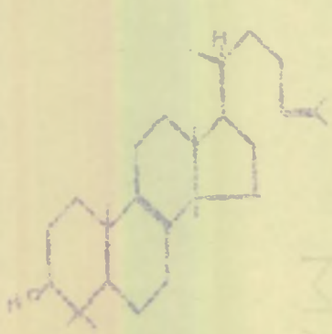
(XXVIII)

(v) Tetracyclic triterpenes.

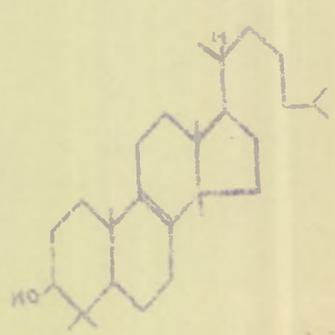
The group of tetracyclic triterpenes includes a number of C-30 alcohols and C-31 acids. The two main families in this class of compounds are lanosterol and euphol. Most of the members of the two groups appear to be structurally similar, but the major points of difference lie in their stereochemistry.

The Lanosterol Group

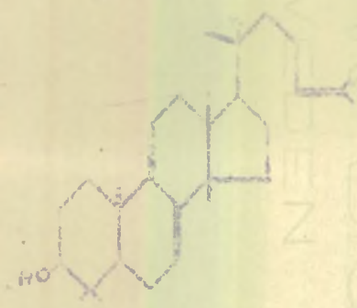
Isocholesterol, considered to be the second pure product of the non-saponifiable fraction of the wool-fat was separated by Ruzicka and his collaborators[59] into the following four substances: (XXIX), (XXX), (XXXI), (XXXII) which differed merely in the number and position of double bonds.



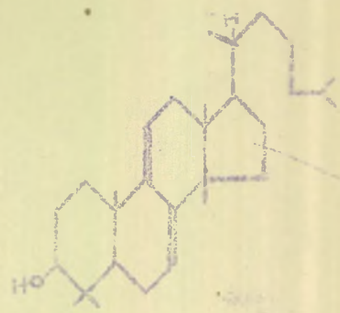
(XXIX)
Lanosterol



(XXX)
dihydrolanosterol

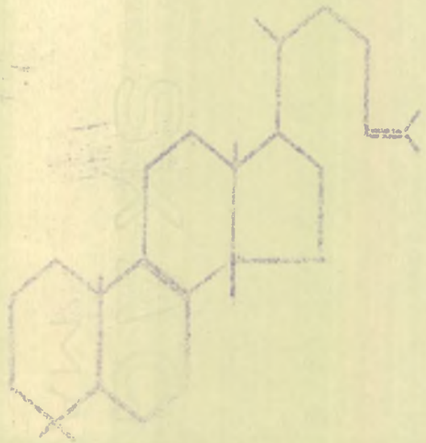


(XXXI)
Agnosterol



(XXXII)
dihydroagnosterol

The parent hydrocarbon was therefore named as lanostane[60] (XXXIII) and the two important members (XXIX), (XXX) were accordingly named, lanostadienol (lanosterol) and lanosterol (dihydrolanosterol).



(XXXIII)

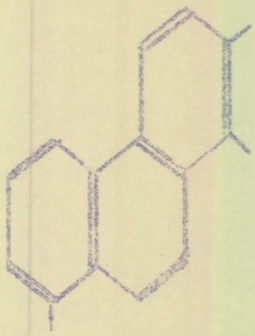
Lanostadienol ($C_{30}H_{50}O$)

The presence of two double bonds in lanostadienol was indicated by titration with perbenzoic acid[61]. A compound with a molecular formula $C_{30}H_{50}O$ and carrying two ethylenic linkages must have a tetracyclic structure. Hydrogenation in presence of platinum catalyst gave lanostenol (dihydrolanosterol), which was still found to contain one double bond proving that the two double bonds were of unequal reactivity.[60,62]

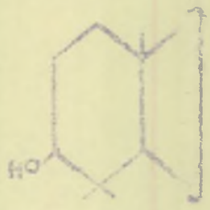
The secondary nature of the hydroxyl group in lanostadienol was proved by Cr O_3 oxidation, when a ketone was obtained.

Selenium dehydrogenation experiments on lanostadienol gave 1:2:8-trimethyl phenanthrene (XXXIV).[63] The characteristic dehydrogenation products of pentacyclic triterpenes or steroidal sapogenins could b not be obtained, indicating a different carbon skeleton of this series of compounds.

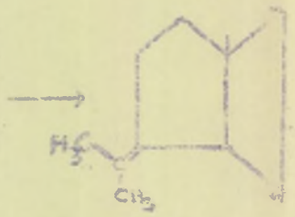
Lanostenol (XXXV) when dehydrated with P Cl_5 gave isolanostadiene (XXXVI) showing the presence of the hydroxyl group in lanosterol adjacent to the carbon atom carrying a gem dimethyl group. Since this reaction is analogous to those in pentacyclic triterpenes, the same ring system is also present in lanostenol.[64]



(XXXIV)



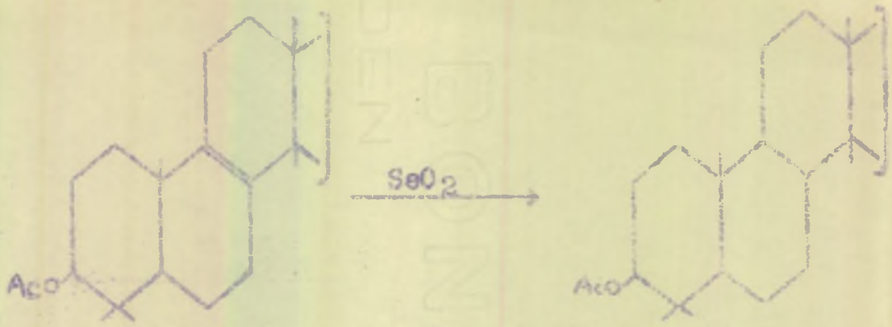
(XXXV)



(XXXVI)

The formation of acetone as a result of oxidation of lanostadienol derivatives carrying the reactive double bond showed the presence of an isopropylidene group in these compounds.[59,65]

The study of the infra red spectra of lanostenol derivatives indicated that the double bond was tetrasubstituted.[66] This was further confirmed by selenium dioxide oxidation of lanosteryl acetate (XXXVII) when a heteroannular diene (XXXVIII) showing the characteristic ultra-violet absorption was obtained.[67] The position of the double bond in lanostenol at C 8-9 was proved by the characterisation of a number of CrO_3 oxidation products of lanosteryl acetate.

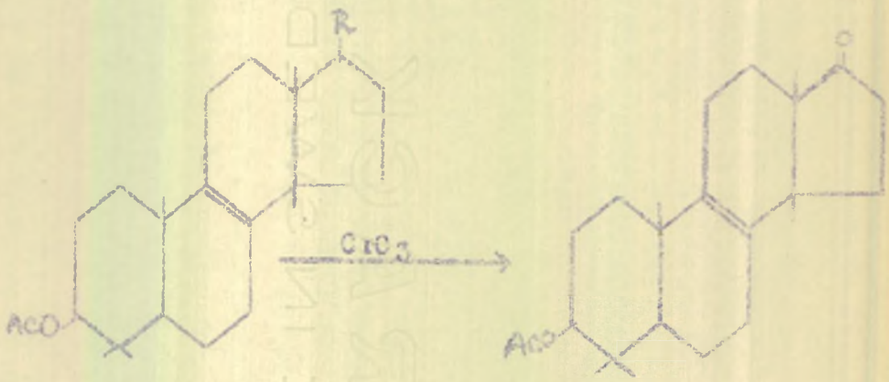


(XXXVII) (XXXVIII)

The formation of 1:2:8-trimethyl phenanthrene (XXXIV) by selenium dehydrogenation coupled with the fact that a greater yield of the above mentioned hydrocarbon was obtained from lanostene (XXXIX) as compared to that from the corresponding alcohol provided evidence for the presence of the methyl groups at C-13 and C-14. This gave the partial structure (XL) for lanostadienol. This also showed the points of attachment of ring D, the size of which was yet unknown.[67]

(XXXIX) (XL)

The vigorous chromic acid oxidation of lanostenyl acetate (XLI) resulted in the complete removal of the side chain, and yielded a ketone (XLII) showing a band at 1745 Cm^{-1} indicating the presence of a carbonyl group in a five membered ring and thus proved the size of ring D in lanostenol.[68]



(XLI) (XLII)

Structure and position of the side chain.

The formation of a C-8 aliphatic ketone during the oxidation of lanostadienol was reported by a number of workers. It was identified as methyl heptane-2-one by Barton and collaborators.[69] This provided sufficient evidence that the side chain of lanostadienol was comprised of 8 carbon atoms. The presence of an iso-propylidene group was indicated by the formation of

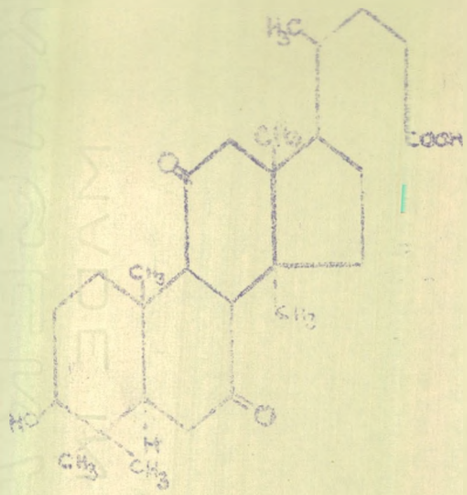
acetone as a result of oxidation. It was further shown that when the reactive double bond was reduced (lanostenol), the oxidation did not yield acetone. This fact provided a conclusive evidence that the reactive double bond was present at C 24-25.

By stepwise degradation, Ruzicka and coworkers[70] were able to give the following structure (XLIII) to the side chain of lanostadienol which had an identical arrangement of atoms as present in the side chain of cholesterol.

(XLIII)

The attachment of the side chain at C-15 or C-13 rather than at C-17 was suggested by Ruzicka and collaborators,[70] as this would have been in conformity with the isoprene rule. Barton,[69,71] however, preferred C - 15 as the point of attachment for the side chain in order to explain the lesser reactivity of the ketone as compared to the C-17 ketone in the androstane series. The attachment of the side chain at C-17 was however, finally established

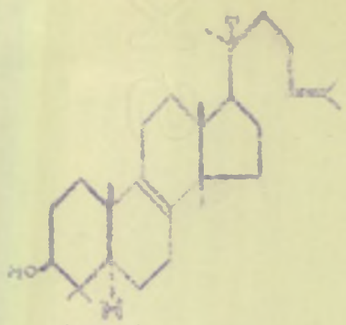
by Ruzicka and collaborators[72] who carried out a thorough investigation of the saturated C_{27} diketo acid (XLIV) obtained from lanosterol.



(XLIV)

Shortly before the chemical proof for the point of attachment of the side chain was given by Ruzicka, X-ray diffraction experiments with lanosteryl iodoacetate revealed the complete structure and the stereochemistry of lanostenol.[73]

The conversion of cholesterol to 14-methyl cholestanol[74] and lanostenol[75] conclusively proved the structure of lanostadienol (XLV) the most important member of the series.



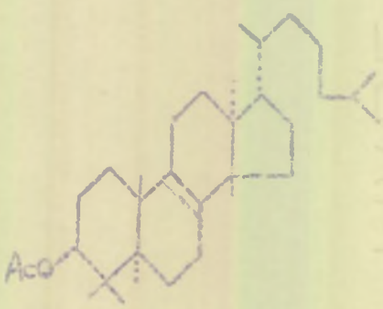
(XLV)

Euphol group

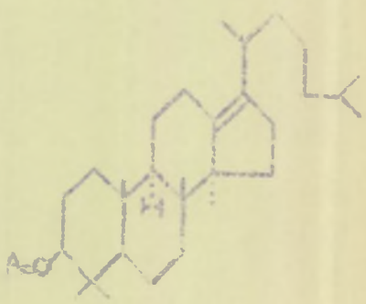
Euphol is the parent substance of the second main group of tetracyclic triterpenes. It was first isolated by Newbold and Spring[76] from Euphorbium resin. Since then its isolation from a number of Euphorbia species has been reported.

It was shown by Swiss workers[77,78] that the side chain of euphol was identical with that of lanostadienol. Warren and coworkers[79] showed the formation of 1:2:8-trimethyl phenanthrene as a result of selenium dehydrogenation of euphol. The same compound was obtained from lanostenol on similar treatment, which proved that the structure of rings A, B and C was identical in both the cases. The position of the double bond at C 8-9 was indicated by the formation of selenium dioxide and chromic acid oxidation products[80,81,82] analogous to those in the lanostane series.

An important difference existing between lanostenol and euphol was in the formation of acid isomerisation products.[83] In the case of lanostane series, acid isomerisation results in the shifting of the double bond from C 8-9 (tetra-substituted) to C 7-8 (trisubstituted). Whereas, in the case of euphane series, the isomerisation product shows the presence of a tetra-substituted double bond, exocyclic to only one ring. Since, the number of angular methyl groups in both the series are same, it is evident that euphol has angular methyl groups at C-13 and C-14 and during isomerisation one of the methyl groups migrates to a new position [84] (XLVI ⟶ XLVII).

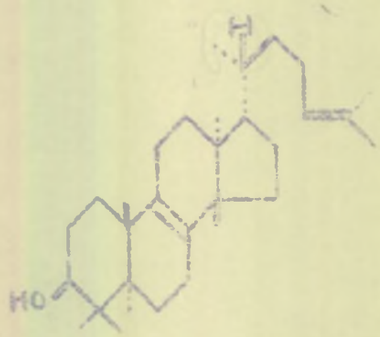


(XLVI)



(XLVII)

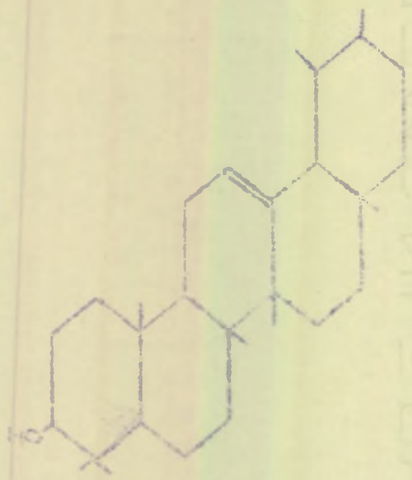
Extensive work on the isomerisation carried out by Halsall,[85] Ruzicka,[86] Jeger[82] and their coworkers resulted in the complete elucidation of the structure and stereochemistry of euphol (XLVIII). Euphol differs from Lanostadienol only in the opposite configuration at C-13, C-14 and C-17 and has the following structure (XLVIII).



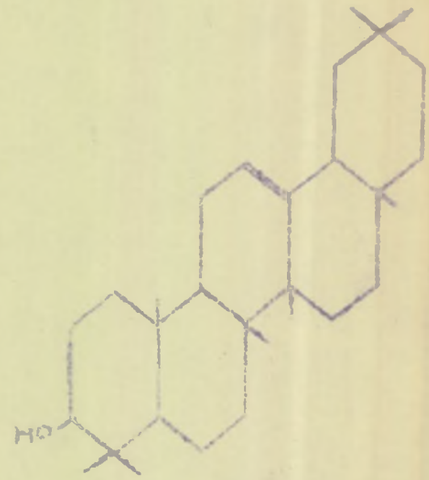
(XLVIII)

Pentacyclic Triterpenes

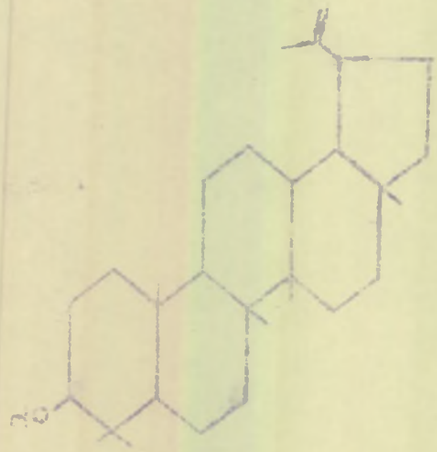
On the basis of the reactivity of the double bond, formation of bromolactone, the infra-red spectra, molecular rotation, and certain other factors, the pentacyclic triterpenes have been divided into the following four groups, named after the key compound of each series. (i) α-amyrin (XLIX), (ii) β-amyrin (L); (iii) Lupeol (LI); (iv) Friedelin (LII).



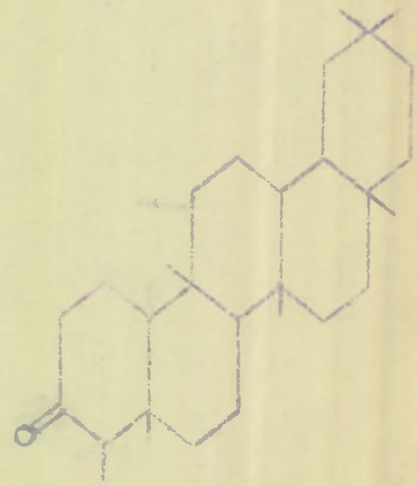
(XLIX)



(L)



(LI)



(LII)

The double bond in the two amyrins and lupeol group has been found to be of very different reactivity. It has been found to be the most reactive in lupeol group, less reactive in the β-amyrin group and least reactive in the case of α-amyrin group.

Jeger[87], on the basis of the comparative reactivity of the double bonds, has tabulated the following results.

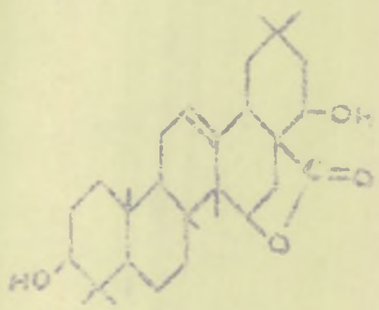
T A B L E I

Type	Catalytic hydrogenation.	Oxidation by peracids.	Action of osmium tetraoxide.	Oxidation by selenium dioxide.
Lupeol Hetero-betulin	hydrogenated derivative.	oxides	diols	β-unsaturated aldehyde
β-amyrin	--	Ketonic derivatives.	--	compounds with chromophores -C-C=C-C(=O)-C=C
α-amyrin	--	--	--	--

While the comparative reactivity of the double bonds in the three groups of pentacyclic triterpenes is generally in accordance with the above results, yet there are exceptions as well.

In the case of dumortierigenin[88] (LIII) a member of the β-amyrin group, it has been noted that its diacetate does not react with selenium dioxide in glacial acetic acid solution under conditions where all known triterpenes of the β-amyrin series are converted

to $\Delta^{11\text{-}13(18)}$-diene.[89]



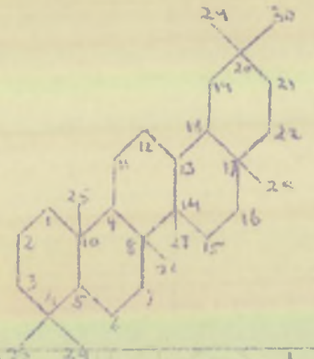
(LIII)

The reaction of N-bromosuccinimide[90] with α- and β-amyrin groups has been reported as another diagnostic tool in differentiating between the members of the amyrin series. In the case of α amyrin, a homoannular $\Delta^{9(11),12}$-diene is formed, while a $\Delta^{9(11),12,18}$-triene is formed in the β-amyrin series. The validity of the above observations has been questioned by Djerassi[91] proving that this reaction is very dependent on the reaction conditions employed, and can not be used as a safe means of differentiation between α- and β-amyrins.

The following three tables give the well known members of the three series which show their close relationship.

T A B L E II

β-amyrin (Oleanane) series



Name	Formula	Genin m.p.	$[\alpha]_D$	Acetate m.p.	$[\alpha]_D$	Methyl ester m.p.	Source
β-amyrin	3-hydroxy Δ^{12-13} oleanene	193-200,	+ 89	241	+ 85.1	-	Manila elemi resin.
Erythrodiol	3-28 dihydroxy Δ^{12-13} oleanene.	232	+ 75.4	187	+ 59.4	-	as monostearat in Erythroxylon novogranatense
Germanicol	3-hydroxy Δ^{18-19} oleanene	180-81,	+ 7	279-80,	+ 19	-	Latex of Lactuca Virosa
Longispino-genin	3;16;28trihydroxy Δ^{12-13} oleanene	247-49,	+ 55	218-21,	+ 73	-	Lemaireocereus longispinus
Manila acid	3;16 dihydroxy Δ^{12-13} oleanene	220-21,	+ 60	205-4,	+ 56	-	Manila elemi resin
Treleasegenic acid	3;21,30 trihydroxy Δ^{12-13} oleanene 28-oic acid	280-90		270-75	+67	282-26 +77	Lemaireocereus treleasei

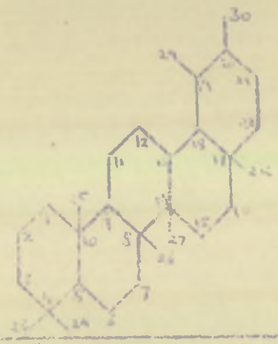
Name	Formula	Genin m.p.	Genin $[\alpha]_D$	Acetate m.p.	Acetate $[\alpha]_D$	Methyl ester m.p.	Source
Echinocystic acid	3β, 16α dihydroxy Δ^{12-13} oleanene-28-oic acid	305-12 decomp.	+40.6 (EtOH)	272-76,	-14.6 (EtOH)	213-15, +37.1 (EtOH)	Echinocystis fabacea
Hederagenin	3,23 dihydroxy Δ^{12-13} oleanene 28-oic acid	327-29,	(Pyridine)	172-74,	+61	240 +20.9	Hedera Helix
Morolic acid	3 hydroxy Δ^{18-19} oleanene-28 oic acid	273	+33	256-57	+42	228-29 +26	Mora Excelsa
Oleanolic acid	3 hydroxy Δ^{12-13} oleanene-28-oic acid	310	+80	268	+74.5	198-200 +75	Olea europaea
Siaresinolic acid	3,19 dihydroxy Δ^{12-13} oleanene 28-oic acid	292			-	182 +44.9	Siam and Sumatra gum benzoin
Sumaresinolic acid	3,6-dihydroxy Δ^{12-13} oleanene 28-oic acid	298-99	+51.6(alc.)		-	220-21 +53.5	Sumatra gum benzoin
Gypsogenin	3-hydroxy 23-aldehyde Δ^{12-13} oleanene 28-oic acid	269-70,		188-89,	+79	-	Saponaria levantica
Quillaic acid	3β, 16α dihydroxy 23-aldehyde Δ^{12-13} oleanene 28-oic acid	292-93	+53.1 (Pyridine)	250		222-23 +40.5 (Pyridine)	Quillaja bark

Name	Formula	Genin m.p.	Genin $[\alpha]_D$	Acetate m.p.	Acetate $[\alpha]_D$	Methyl ester m.p.	Source
Primula genin A	3:16:28trihydroxy Δ^{12-13} oleanene	249-50,	+ 58	159-60,	- 9.7	-	Primula officinalis
Soya-sapogenol C	3:23dihydroxy $\Delta^{12-13,15-16}$ oleadiene	239,	+ 71	198		-	Soya beans
Soyasapogenol A	3:23:15:16-tetrahydroxy Δ^{12-13} oleanene.	321,	+ 102	232,	+ 86	-	Soya beans
Soyasapogenol B	3:23:16 trihydroxy Δ^{12-13} oleanene	260,	+ 92	180	+ 83	-	Soya beans
Soyasapogenol D	3,23 dihydroxy 16,26 oxo, Δ^{12-13} oleanene.	298-99 (in C_2H_5OH)	- 61	191	- 45	-	Soya beans
Arjunolic acid	2,3,23-trihydroxy Δ^{12-13} oleanene -28-oic acid	337-40,	+63.5	-		248 +66 in EtOH	Terminalia Arjuna
Bassic acid	2:3:23 trihydroxy $\Delta^{5-6,12-13}$ oleadiene-28-oic acid	316	+82.4(Pyr)	-		214-15 +84	Bassia species
α-Boswellic acid	3α:hydroxy Δ^{12-13} oleanene 23-oic acid	289	+ 111	242	+ 65	214 +115	Boswellia cateri
Cochalic acid	3,16 dihydroxy Δ^{12-13}-oleanene-28-oic acid	303-306 (dioxane)	+ 58	194-96		192-94 +55	Myrtillocactus Cochal.

Name	Formula	Genin m.p.	$[\alpha]_D$	Acetate m.p.	$[\alpha]_D$	Methyl ester m.p.	Source
Glycyrrhetic acid	3-hydroxy,11-keto Δ^{12-13} oleanene 30-oic acid	300-304, 287-93	+163	309-13,	+145	259	Sweet Liquorice
Machaeric acid	3-hydroxy 21-keto Δ^{12-13} oleanene 28-oic acid	309-12,	+ 20	-		197-98 +23	Machaerous gummosus
Gummosogenin	3,16 dihydroxy 28 al Δ^{12-13} oleanene	251-52	+28	219-21	+66	-	Machaerous gummosus
Dumortierigenin	28-15 lactone of 3β,15β,22α trihydroxy Δ^{12-13} oleanene 28-oic acid	292-95,	-18.6	318-21	-10	-	Lemaireocereus dumortieri
Machaerinic acid	3,21 dihydroxy Δ^{12-13} oleanene 28-oic acid	286-88,	+82.4	258-60,	+88	232-34 +76	Machaerous gummosus and Albizzia Procera Benth
Queretaroic acid	3,30 dihydroxy Δ^{12-13} oleanene 28-oic acid	318-23		292-95,	+82	223-24 +87	Lemaireocereus queretaroensis
Myrtillogenic acid	3,16,28 trihydroxy Δ^{12-13} oleanene 29-oic acid	288-93,	+84	259-63	+65	249-50 +85	Myrtillacactus
Chipechipigenin	3,16,22,28-tetrahydroxy Δ^{12-13} oleanene	321-23,	+43	280-82,	+26	-	Lemaireocereus chipechipe

Name	Formula	Genin m.p.	$[\alpha]_D$	Acetate m.p.	$[\alpha]_D$	Methyl ester m.p.	Source
Medicagenic acid	2,3-dihydroxy Δ^{12-13} oleanene 23,28-dioic acid	349-50,	+111	210-12,	+94	221.5-24 +93.5	Medicago sativa
Terminelic acid	2,3,6,23(24)tetra- hydroxy Δ^{12-13} oleanene 28-oic acid	347	+42	217	-12.5	165-68 +40	Terminalia ivorensis
Barringtogenol	2,3,23,28 tetra- hydroxy Δ^{12-13} oleanene	290-91,	+18	269-70,	+15	-	Barringtonia racemosa
Barringtogenic acid	2,3 dihydroxy Δ^{12-13} oleanene 23,28-dioic acid	332-34, decomph.	+72	334-36, discomp.	+45	264-66 +63	Barringtonia racemosa
Entagenic acid	3,(21,22)or(15,16) trihydroxy Δ^{12-13} oleanene-28-oic acid	310-15 (decomp)		188-9		243-5	Entada phaseoloides
Glabric acid	3,18 dihydroxy 11-keto Δ^{12-13} oleanene-30-oic acid	329-33	-26	308-309	+38	277-80 +16	Liquorice root
Albigenic acid	3β 16α dihydroxy Δ^{13-18} oleanene 28-oic acid	246-48	-13 (EtOH)	-		225-26 -10	Albizzia lebbek Benth
A-Barrigenol	3,15,16,27,28- pentahydroxy Δ^{12-13} oleanene	300-302,	+4	279-80		-	Barringtonia asiatica
7β-hydroxy-A, barrigenol	3β,7β,15α,16β, 27,28-hexahydroxy Δ^{12-13} oleanene	208-10 (Diox)	+37	186-87.5	-88	-	Leaves of Pittosperum undulatum

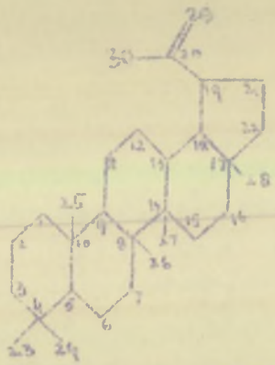
T A B L E III



α-amyrin-ursane series

Name	Formula	Genin M.p.	Genin $[\alpha]_D$	Acetate M.p.	Acetate $[\alpha]_D$	Methyl ester M.p.	Source
α-Amyrin	3-hydroxy Δ^{12-13} ursene	186.5-187	+91.6 (in C_6H_6)	227	+83.3	-	Manila elemi-resin.
Brein	3,21(or 22)dihydroxy Δ^{12-13} ursene	215-17,	+65.5 EtOH	196		-	Manila-elemi-resin
Uvaol	3,28-dihydroxy Δ^{12-13} ursene	233,	+74.4	157-59,	+54 (Benzene)	-	Arctostaphylos Uva-ursi
Asiatic acid	2,3,23 trihydroxy Δ^{12-13} ursene-28-oic acid.	300-305	+55 EtOH	-		325	+52 Centella asiatica
β-Boswellic acid	3α-hydroxy Δ^{12-13} ursene 24-oic acid	238	+116.5	271	+69	191	+116 Boswellia carteri
Quinovic acid	3 hydroxy Δ^{12-13} ursene 27,28 dioic acid.	298	+87 (Pot. salt)	284		173-74 +116	Cinchona bark
Ursolic acid	3 hydroxy Δ^{12-13} ursene,28 oic acid	286-7	+ 67.5 in N-alc.NaOH	289-90	+ 61.5	170.5-171.5 +58(Pyr)	Arctostaphylos Uva-ursi
Sauerenol	3 hydroxy Δ^{7-8} ursene	207-208	-30	293-94	-37		Acronychia baueri

Table IV



Lupane Series

Name	Formula	Genin M.P.	Genin $[\alpha]_D$	Acetate M.P.	Acetate $[\alpha]_D$	Methyl ester M.P.	Source
Betulin	3,28-dihydroxy Δ^{20-29} lupene	261	+20(Pyr.)	233-24,	+22	-	Betula-alba bark
Betulinic acid	3-hydroxy Δ^{20-29} lupene 28-oic acid	316-18	+7o	290-92	+20.1	224-5 +5.0	Cornus florida bark
Lupeol	3-hydroxy Δ^{20-29} lupene	215-16	+27.2	220	+47.3	-	Lupinus albus
Stellatogenin	28→19 lactone of 3,19,20 trihydroxy lupane-28-oic acid	317-19	+36	327-30	+53	-	Lemaireocereus stallatus
Thurberogenin	28→19 lactone of 3,19 dihydroxy Δ^{20-29} lupene-28-oic acid	293-95	+11	249-52	+45	-	Lemaireocereus thurberi
Melaleucic acid	3 hydroxy Δ^{20-29} lupene 25,28-dioic acid	363-64		-		204	Melaleuca species
23-hydroxy betulin	3,23,28 trihydroxy Δ^{20-29} lupene	259-60	+24.6	-		-	Sorbus aucuparia
Lupane 3 ,20 diol	Lupane 3 ,20 diol	239-41	+4	3-Monoacetate 252-56	+16	-	

β - AMYRIN

The following three methods have generally been employed in the elucidation of the structure of these compounds. These methods are discussed below in detail as applied to β-amyrin series.

(i) Dehydrogenation

(ii) Oxidative degradation

(iii) Interconversions.

(i) Dehydrogenation.

The study of the carbon skeleton of the triterpenic compounds based on dehydrogenation results has been extensively utilized by Ruzicka and collaborators.[92] Selenium dehydrogenation of practically all the triterpenes gave 1:2:7-trimethyl naphthalene (Sapotalene) and a number of other naphthalene derivatives, which were identified by comparison with authentic samples obtained by synthesis.

The following compounds are now taken to be the typical dehydrogenation products of the pentacyclic triterpenes.

(a) 1:2:3:4-tetramethyl benzene (XIX)

(b) 2:7-dimethyl naphthalene (XX)

(c) 1:2:7-trimethyl naphthalene (Sapotalene) (XXI)

(d) 1:2:5:6-tetramethylnaphthalene (XXII)

(e) 6-hydroxy-1:2:5-trimethyl naphthalene (XXIII)

(f) 1:8-dimethyl picene (XXIV)

(g) 1:8-dimethyl picene-2 ol. (XXV)

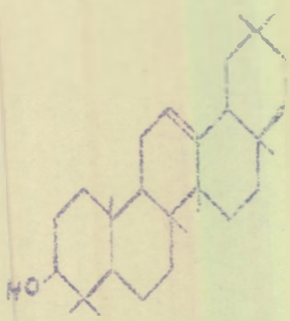
The isolation of 1:8-dimethyl picene (XXIV) proved conclusively that the pentacyclic system of the triterpenes was a reduced picene nucleus, while the isolation of 6-hydroxy 1:2:5-trimethyl naphthalene fixed up the structure of rings A and B with certainty. Rings D and E account for 2:7-di-and 1:2:7-trimethyl naphthalenes, and the naphthol arises from rings A and B.

The formation of (XIX) and (XXII) from rings A and B of the triterpene skeleton is considered to take place by retropinacolic rearrangement involving loss of the hydroxyl group at C-3 by dehydration. The hydroxyl group does not undergo any rearrangement in the formation of naphthol (XXIII) and picenol (XXV).

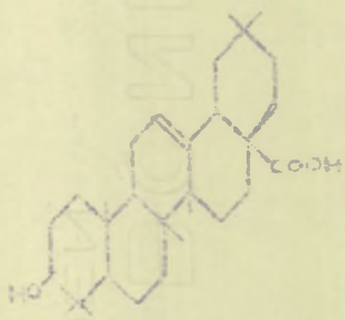
(ii) Oxidative degradation.

The elucidation of the structure of the members of β-amyrin group is mainly based on the experiments performed on oleanolic acid (LIV) and hederagenin (LVI). Oleanolic acid and hederagenin differ from each other only in the fact that in oleanolic acid one COOH group replaces a CH_3 group of β-amyrin (LV) and in hederagenin

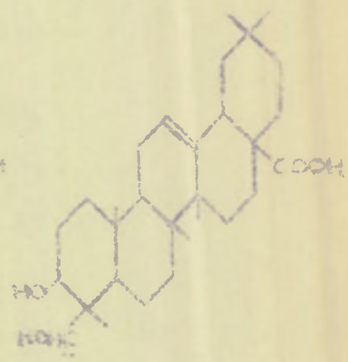
a CH_2OH group replaces one more methyl group of the oleanolic acid.



(LIV)



(LV)

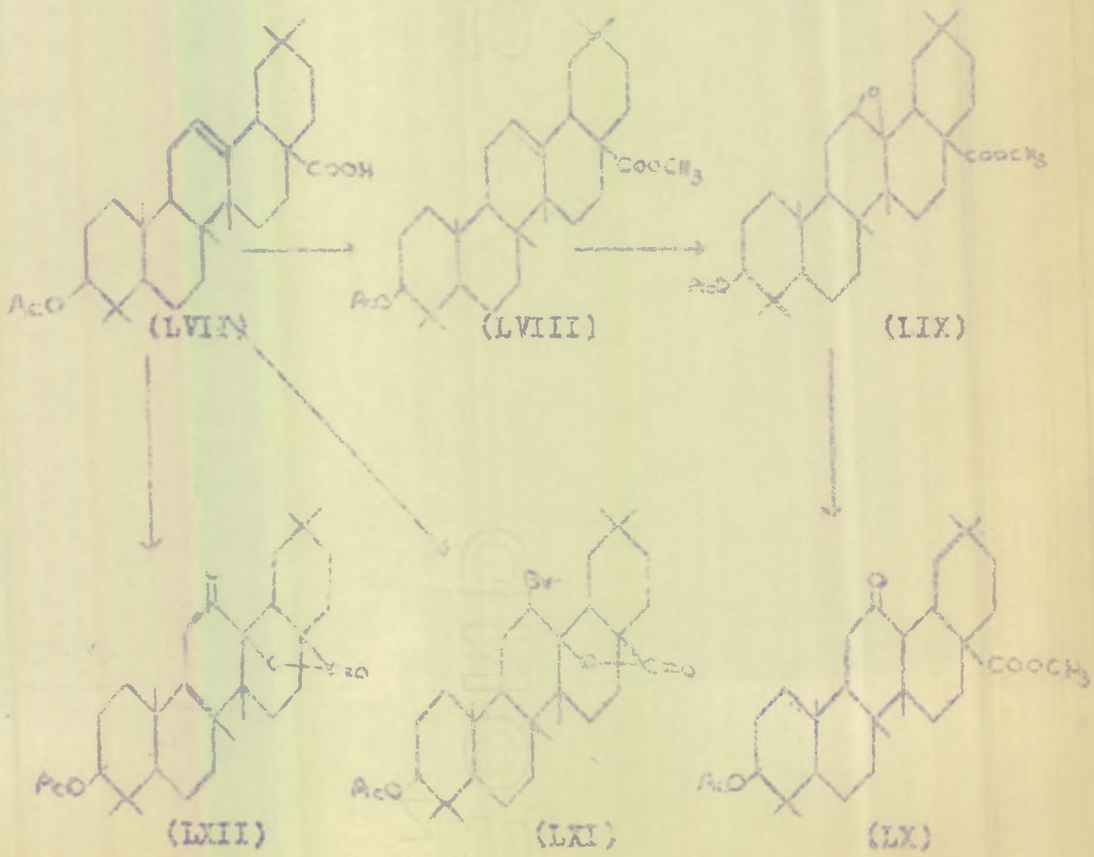


(LVI)

Nature and position of the carboxyl group and the double bond.

The carboxyl group of oleanolic acid (LVII) is very difficultly esterified and once the methyl ester (LVIII) is obtained by the action of diazomethane, it is very difficult to hydrolyse it. This indicates that the carboxyl group is in a hindered position, or tertiary in nature and situated probably at C-17, which is a tertiary carbon atom.

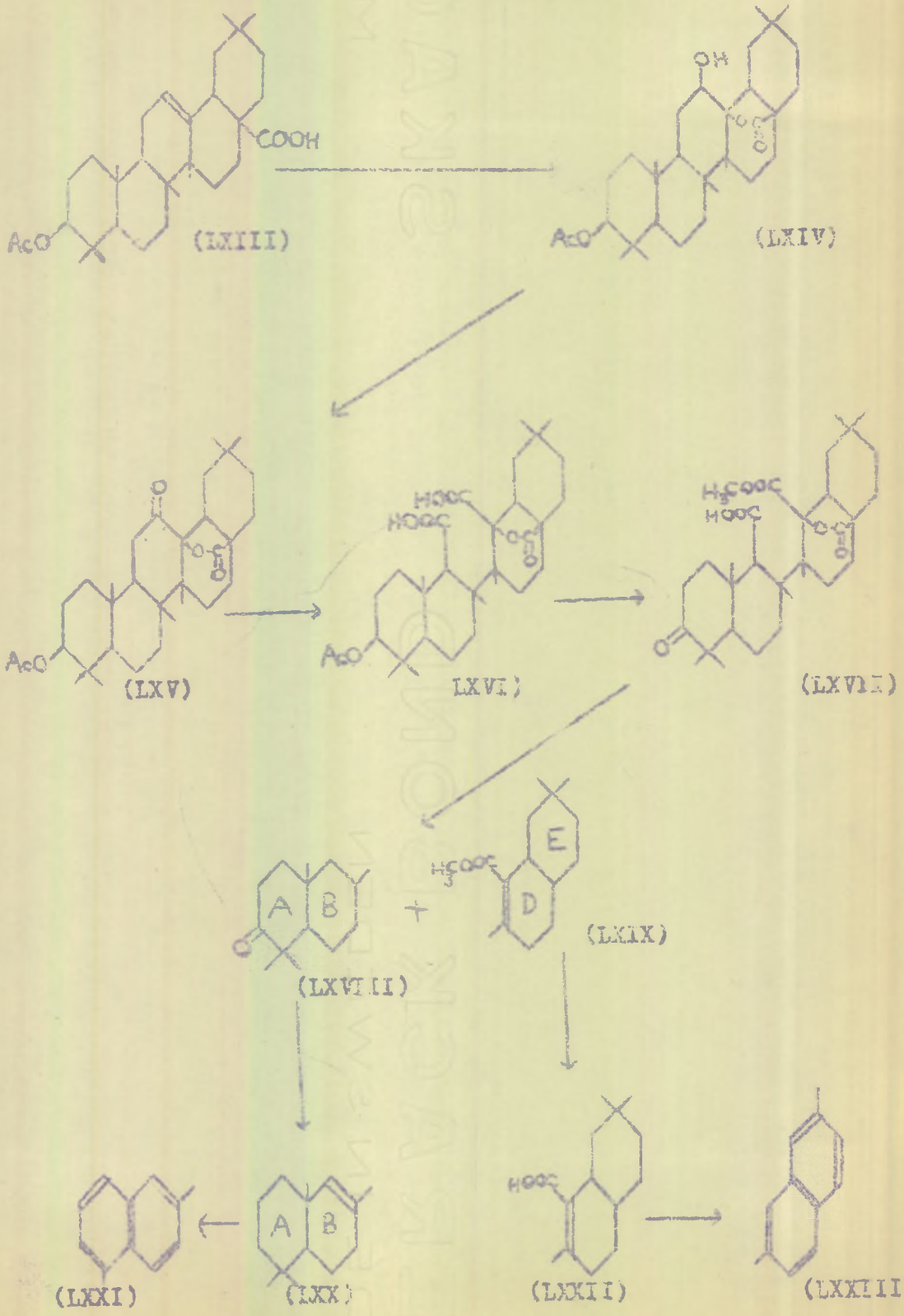
The location of the double bond in clearolic acid presented considerable difficulties due to its inert nature. Catalytic hydrogenation did not succeed in saturating the double bond and its presence was detected only by the production of a yellow colour with tetranitromethane. It however, responded to the treatment with peracids giving an epoxide (LIX) which was subsequently transformed to a ketone (LX). It also readily yielded a bromo- (LXI) and a keto-lactone (LXII).



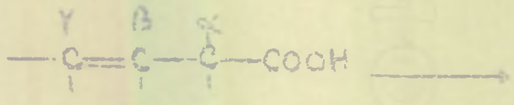
The conclusive position of the double bond at C 12-13 in ring C was established by a series of reactions involving the fission of ring C carried out by Ruzicka and collaborators[93-96].

Hydroxylation of the double bond in acetyl oleanolic acid (LXIII) by CrO_3 followed by further oxidation yielded a hydroxy lactone (LXIV) a ketolactone (LXV) and finally a lactone dicarboxylic acid (LXVI) due to the fission of ring C. The monomethyl ester (LXVII) of the ketolactone dicarboxylic acid on pyrolysis yielded a ketonic (LXVIII) and an ester fraction (LXIX). Reduction of the ketone (LXVIII) to the hydrocarbon (LXX) and subsequent selenium dehydrogenation resulted in the formation of 1:6-dimethyl naphthalene (LXXI). The ester fraction (LXIX) after saponification yielded 2,7-dimethyl-naphthalene (LXXIII) on similar dehydrogenation.

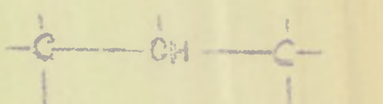
The formation of the ketonic and the ester fractions can be explained very clearly if the double bond is located in ring C.


COOH
AcO
OH
(LXIII)
(LXIV)
(LXV)
(LXVI)
(LXVII)
A
B
E
D
(LXVIII)
(LXIX)
(LXXI)
(LXX)
(LXXII)
(LXXIII)

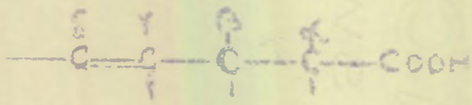
The position of the double bond in $\beta\gamma$ (LXXIV) or $\gamma\delta$ (LXXVI) position with respect to the carboxyl group was indicated by the formation of a five membered lactone ring (LXXV); (LXXVII) without involving the hydroxyl group at position 3[97,98].



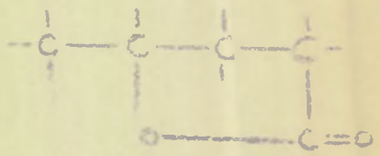
(LXXIV)



(LXXV)

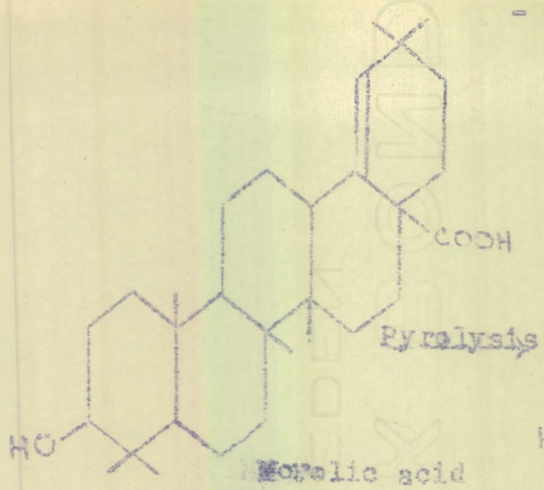


(LXXVI)

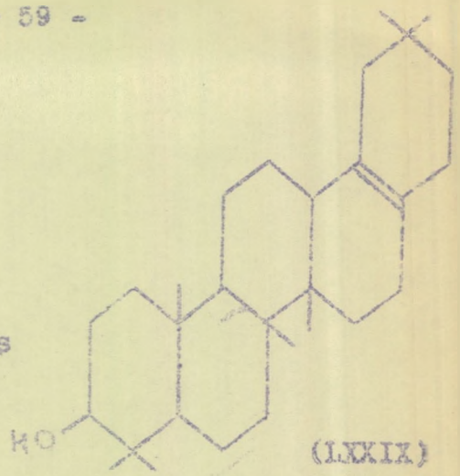


(LXXVII)

The possibility of the presence of the double bond in $\beta\gamma$ position with respect to the carboxyl group was ruled out when oleanolic acid did not undergo decarboxylation on pyrolysis, which is a common feature of $\beta\gamma$ unsaturated acids (LXXVIII). Such decarboxylation is always accompanied by the displacement of the double bond to $\alpha\beta$-position[99] (LXXIX).



Pyrolysis

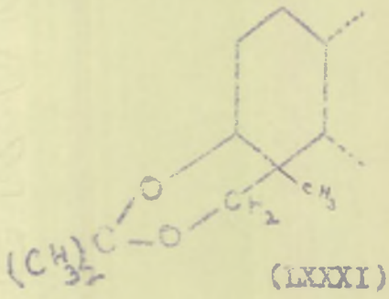


(LXXIX)

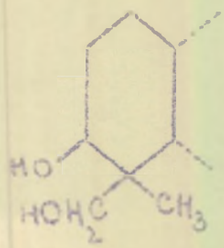
Hederolic acid
(LXXVIII)

Location of the primary alcoholic group in hederagenin:

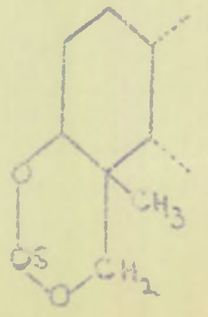
Hederagenin (LXXX) on treatment with acetone in acid medium readily forms an acetonyl derivative (LXXXI)[100]. It also reacts with thionyl chloride to form a cyclic sulfite of the acid chloride (LXXXII) thus showing that the two hydroxyl groups were in 1:3-position.



(LXXXI)

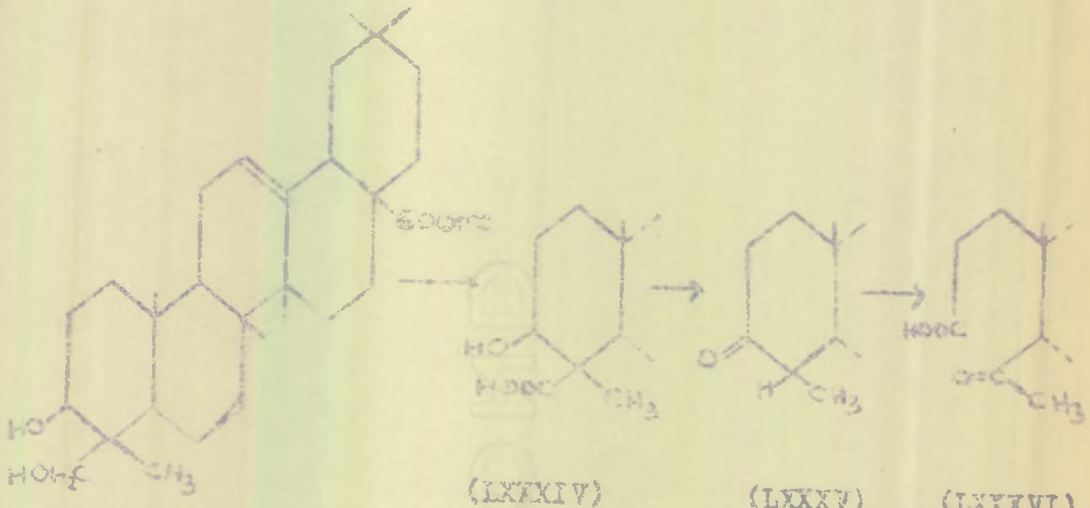


(LXXX)



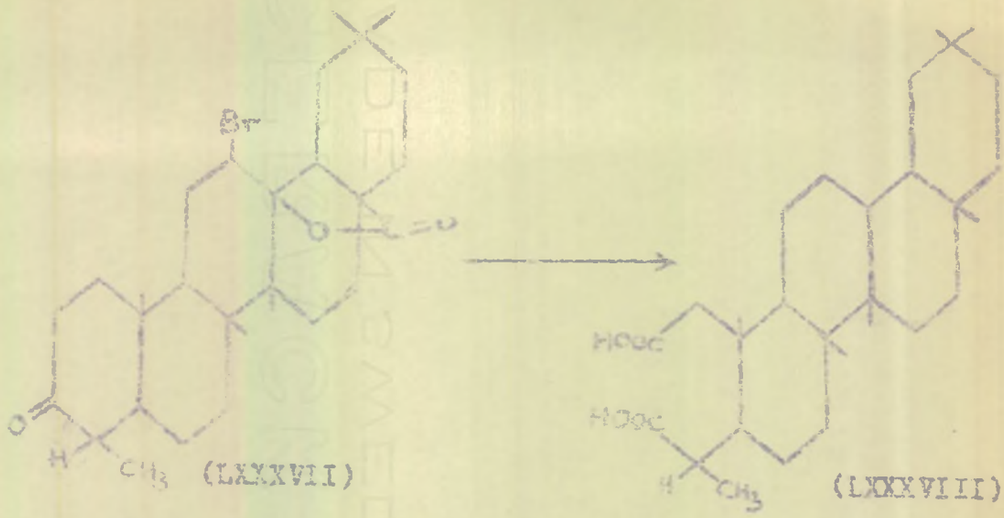
(LXXXII)

The CrO_3 oxidation of the methyl ester of hederagenin (LXXXIII) results in the formation of a neutral ketone (LXXXV) and a keto acid (LXXXVI) both having one less carbon atom than the original ester[100,101]. This could be explained only by the easy decarboxylation of the β-keto acid formed by the oxidation, if the hydroxy methyl group is situated at position 4.



(LXXXIII) (LXXXIV) (LXXXV) (LXXXVI)

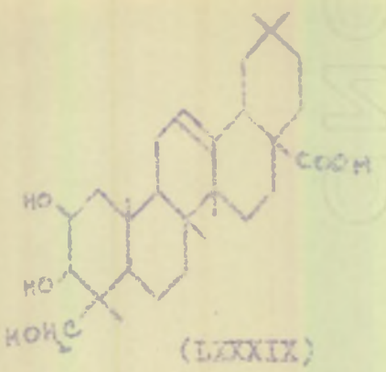
The presence of a methylene group[102] at position 2 can be shown by the formation of a dicarboxylic acid (LXXXVIII)(without loss of any carbon atom) by the oxidation of the ketone (LXXXVII) obtained from the bromolactone of hederagenin.



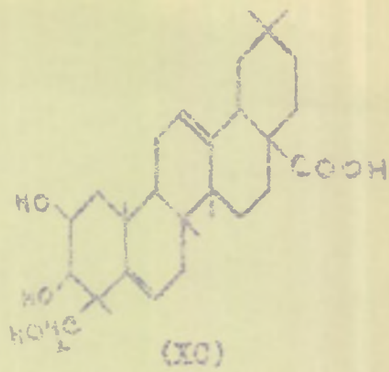
(LXXXVII) (LXXXVIII)

The presence of two hydroxyl groups in the same ring adjacent to each other is fixed up by periodic oxidation, which results in the opening of the ring and giving of an oxidation product[108] containing the same number of carbon atoms. If the two groups are not adjacent to each other, the starting material is recovered unchanged. Therefore, this oxidation is a ready method for discovering the adjacent position of the two hydroxyl groups.[103-106].

Thus in the case of arjunolic acid (LXXXIX)[107] and Bassic acid (XC)[108] the presence of the two hydroxyl groups in the 1:2 position has been fixed up by utilising periodic oxidation method.



(LXXXIX)



(XC)

Peracid oxidation:

An indication of the presence of an ethylenic linkage in a compound is given by a yellow color with tetranitromethane.[48] In certain cases the preparation of an epoxide, by the oxidation of the acid or its derivatives with perbenzoic or perphthalic acid has also been utilized to prove the presence of ethylenic linkage. The epoxide further transforms itself to a ketone (LIX LX). Since α-amyrin derivatives fail to yield an epoxide or a ketone, the peracid oxidation has also been used for differentiating between the two groups.[107]

Interconversions

Due to the identical ground structure of all the triterpenes belonging to a pentacyclic group, a relationship can be very easily demonstrated by simple interconversions of various members. The method consists in the transformation of a functional group to any other suitable group, such as the conversion of -COOH group to

$-CH_3$, or CH_2OH group or the change of an aldehydic group to a methyl group etc.

$$RCOOH \longrightarrow RCOCl \xrightarrow[\text{reduction}]{\text{Rosenmund}} RCHO \xrightarrow[\text{reduction}]{\text{Wolff-Kishner}} RCH_3$$

The use of lithium aluminium hydride has also been made in the conversion of these groups.

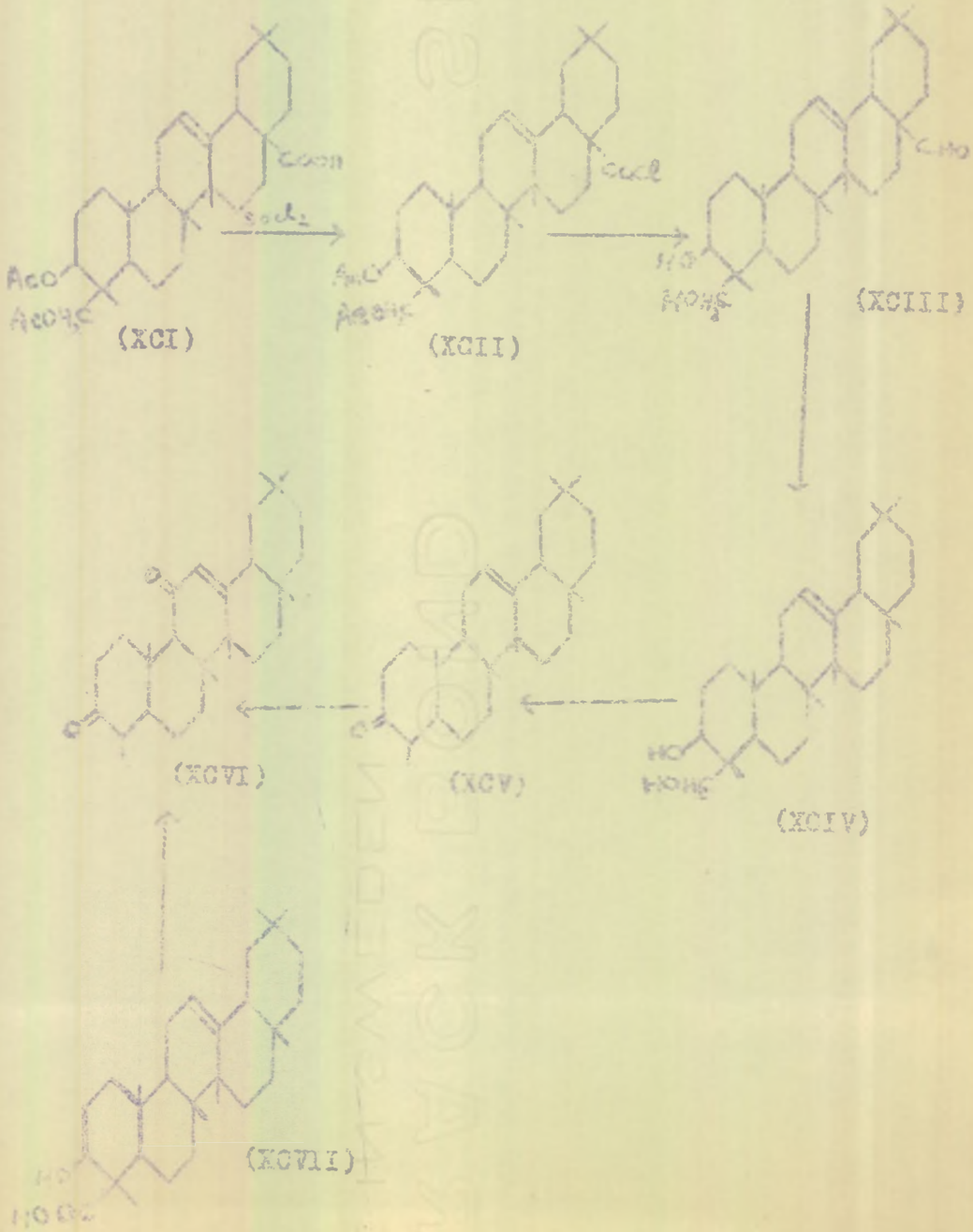
$$\text{R-COOH or R-COO Me or R-CO-Cl} \xrightarrow{LiAlH_4} R\text{-}CH_2OH \xrightarrow{Cr_2O_3} RCHO \downarrow RCH_3$$

$$R\text{-}CH_2OH \xrightarrow[\text{Chloride}]{\text{Tosyl}} R\text{-}CH_2OTS \xrightarrow{LiAlH_4} R\text{-}CH_3$$

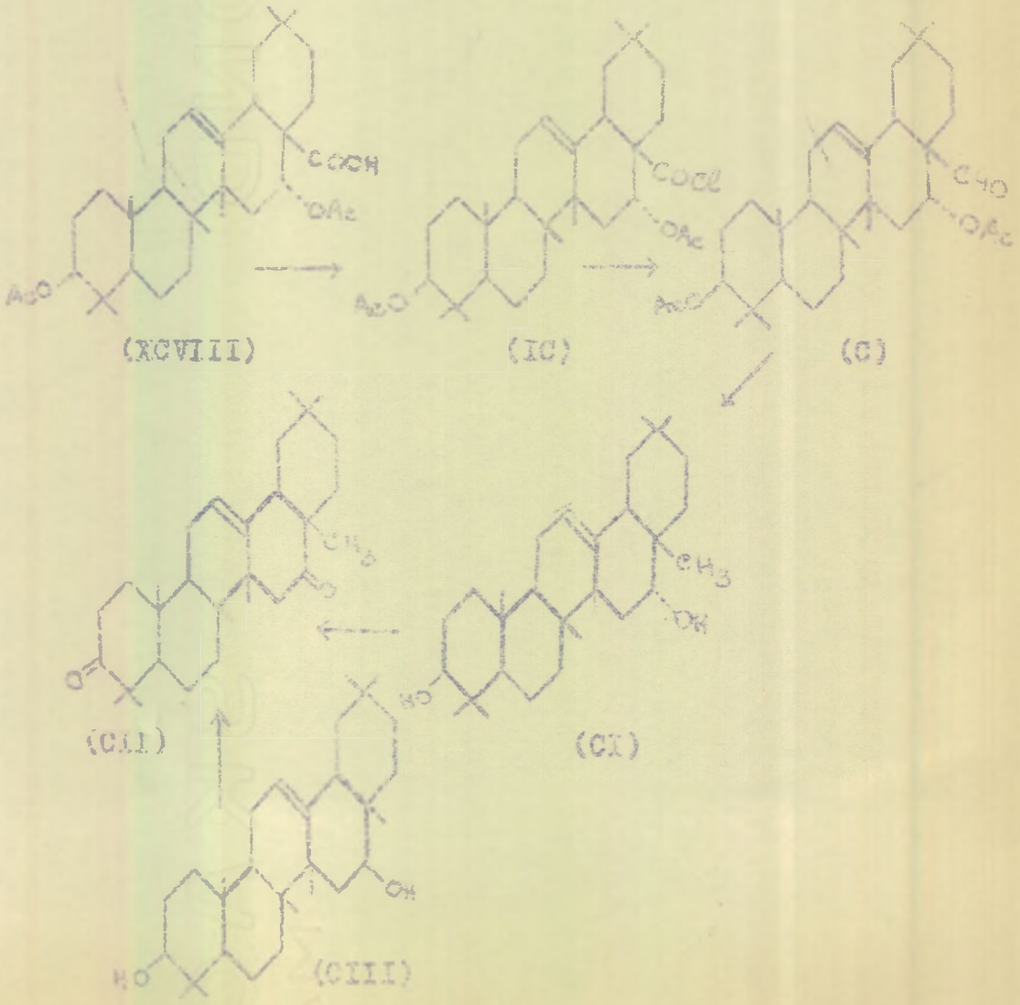
In some cases the complete removal of certain groups is also helpful for establishing a relationship with a triterpene of known constitution.

Ruzicka and Marker[100] have interrelated hederagenin and α-Boswellic acid (XCVII) by preparing the acid chloride (XCII) of hederagenin acetate (XCI), and then subjecting it to Rosenmund reduction which gave the aldehyde (XCIII). The Wolff Kishner reduction of the aldehyde yielded the hederadiol, (XCIV) on further reduction it gave the nor - β-amyrone (XCV) which on chromic acid oxidation furnished the diketone (XCVI). This

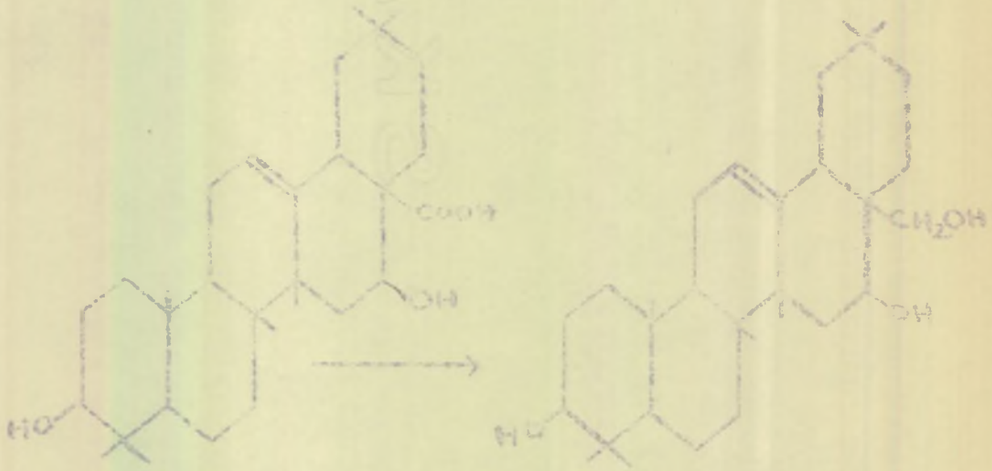
diketone has also been obtained by Ruzicka and Wirz[110] by the oxidation of β-Boswellic acid (XCVII) with chromic acid.



The conversion of the -COOH group in diacetyl echinocystic acid (XCVIII) to a methyl group (CI) through the acid chloride (IC) and aldehyde (C) yielded a diol (CI) which was not identical with maniladiol, because of the different configuration of the -OH group at C-16. However the diketone (CII) obtained from the diol was shown to be identical with the diketone of maniladiol (CIII), and thereby a relationship between the two compounds (XCVIII) and (CIII) was established.

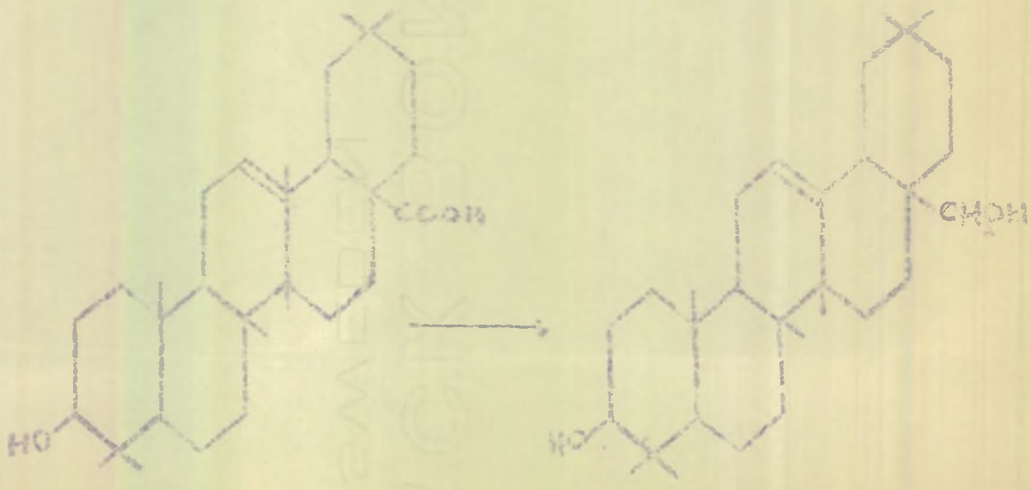


Similarly Primula genin A (CV) has been obtained by reductive methods from echinocystic acid (CIV).



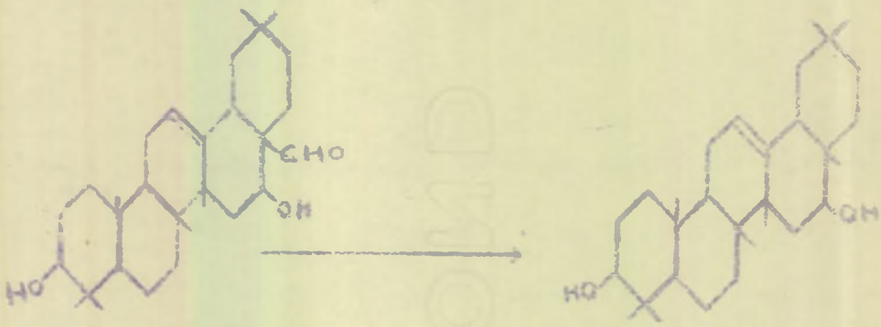
(CIV) (CV)

Lithium aluminium hydride reduction of oleanolic acid (CVI) yields erythrodiol (CVII)[112].

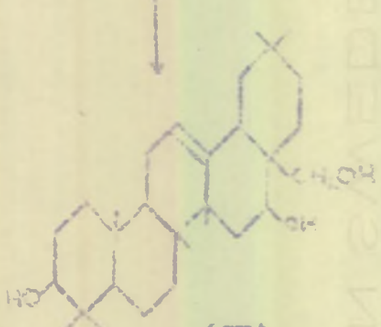


(CVI) (CVII)

Gummosogenin (CVIII) on Wolff-Kishner reduction yields maniladiol (CIX)[113] while Longispinogenin (CX) has been obtained by Lithium aluminium hydride reduction of gummosogenin.[113]



(CVIII) (CIX)



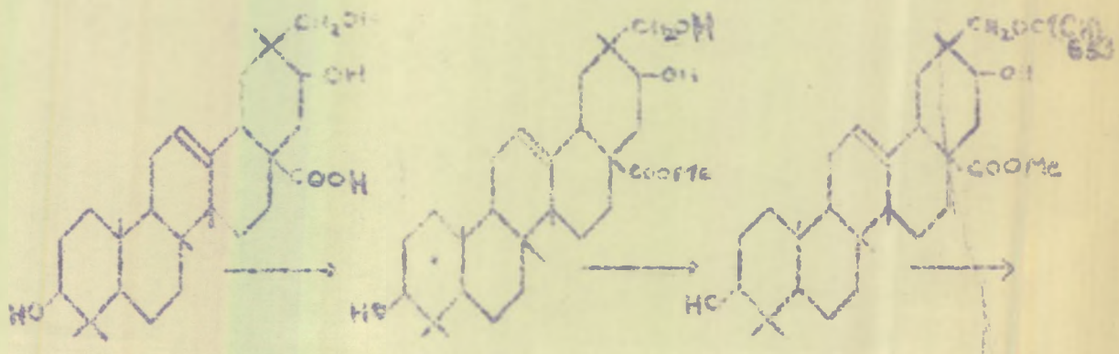
(CX)

The structure of the cactus triterpene trelosso-genic acid[114] (CXI) has successfully been established by inter-relating it with the triternenes of known constitution.

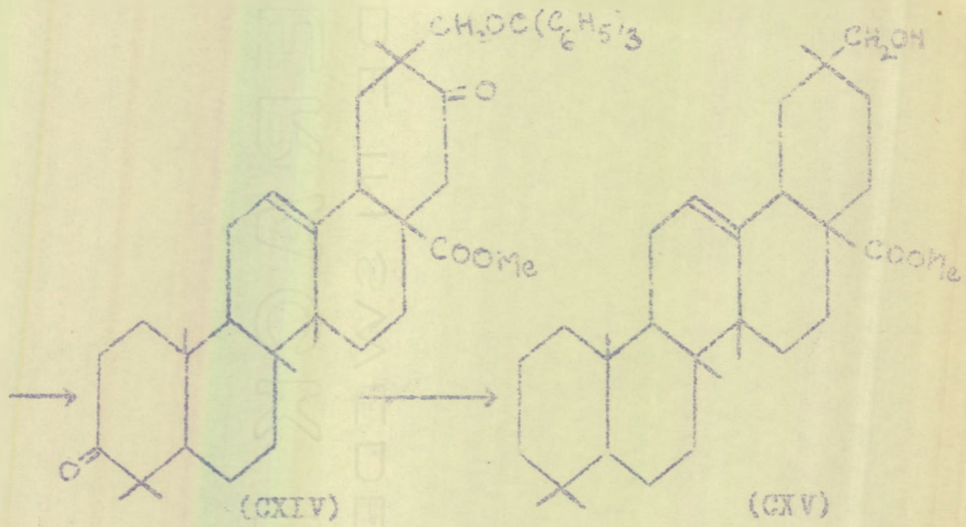
The elemental analysis, the functional group determination, the ready bromolactone formation and the selenium dioxide oxidation results fixed up the acid to

be a trihydroxy monocarboxylic acid belonging to the β -amyrin group with the molecular formula $C_{30}H_{48}O_5$. By analogy to all other triterpenes, one of the hydroxyl group is expected to be attached at C-3 (β).

The treatment of methyl trelesegenate (CXII) with triphenylmethyl (trityl) chloride in pyridine-dioxane solution gave the trityl ether (CXIII) which was characterised as the diacetate, (tritylation reaction etherifies primary hydroxyl groups only)[115]. Oxidation of the trityl ether with chromium trioxide gave the diketo-trityl ether (CXIV), which on Wolff-Kishner reduction, remethylation and trityl ether cleavage gave the monohydroxy methyl ester (CXV) further characterised as the monoacetate.



(CXI) (CXII) (CXIII)

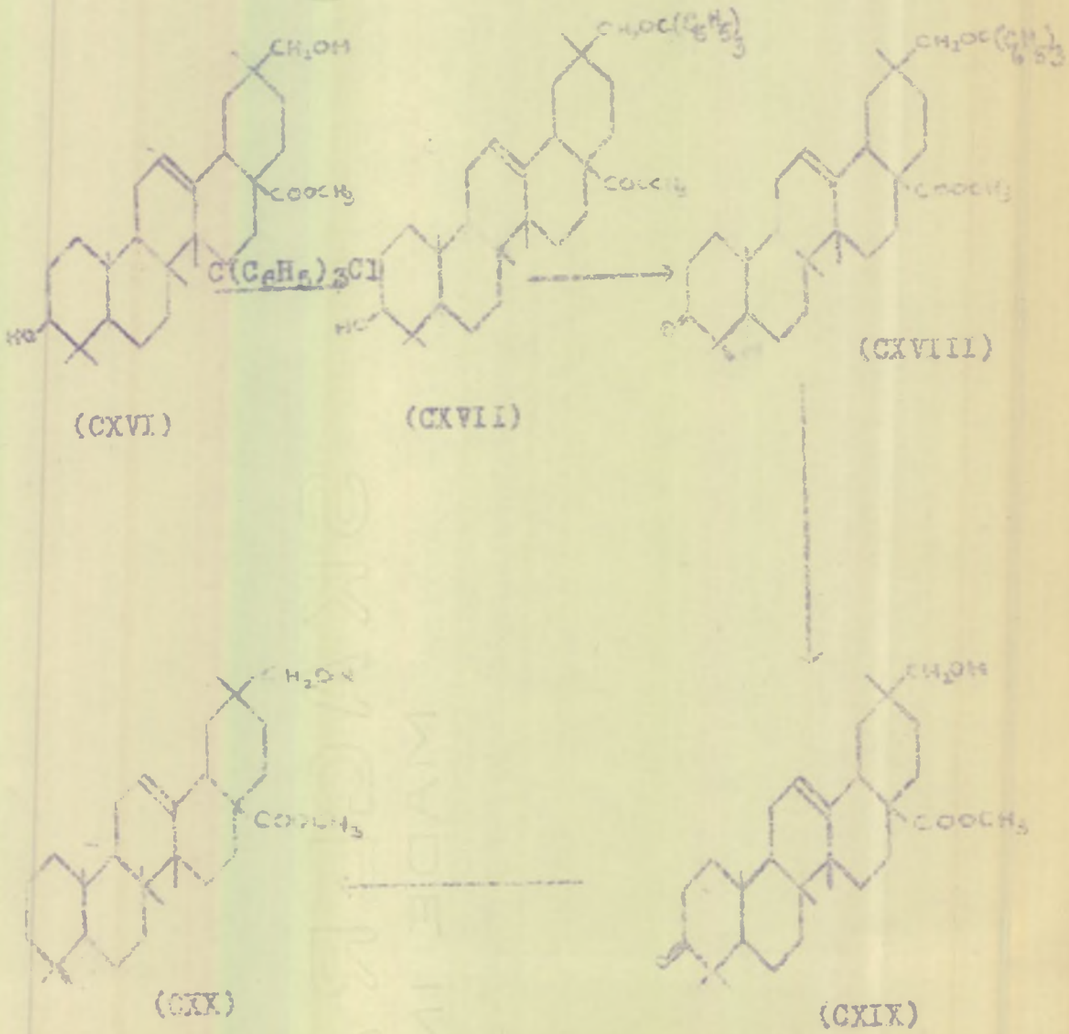


(CXIV) (CXV)

The early work[116] with cactus triterpones showed that oxygenation is only to be expected in rings D and E aside from C-3. The presence of the carboxyl group in treleasegenic acid has already been assumed at C-17 by the ready formation of bromolactone, hence the most likely position for the primary hydroxyl function would be at C-29 or C-30.

In order to establish some relationship between treleasegenic acid and some known triterpene with the OH group at C-29 or 30, the 3-hydroxyl group in methyl queretaroate (CXVI)[117] was removed by first protecting the OH group at C-30 by preparing its trityl derivative and then subjecting it to chromium trioxide oxidation in pyridine solution. The trityl group was removed by boiling with acid. The resulting methyl 3-dehydroqueretaroate (CXIX) on Wolff-Kishner reduction and remethylation gave the

3-deoxoqueretarcate (CXX). This was found to be identical in every respect with the monohydroxymethyl ester (CXV) obtained previously from methyl treleasegenate.

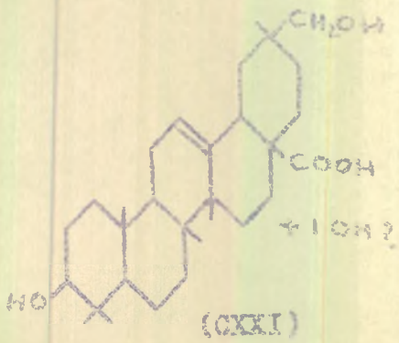


(CXVI) (CXVII) (CXVIII)

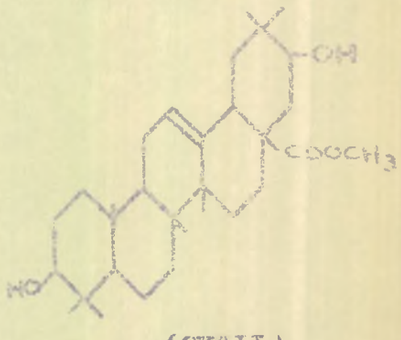
(CXX) (CXIX)

Thus it provided an unambiguous proof for the structure of the trelleasegenic acid (CXXI) as a member of β-amyrin group with a carboxyl group at C-17, two hydroxyl groups at C-30, and C-3, leaving the position of one OH group still to be established.

The surprising ease of saponification of methyl trelleasegenate (CXXIII)paralleling that of methyl machaerinate (CXXII)[118] suggested activation by a γ -hydroxyl group, and this left only positions 15 α , 19 β and 21 β open for consideration. The first possibility was excluded since 15 hydroxyl function can not be acetylated under standard conditions.[119]



(CXXI)

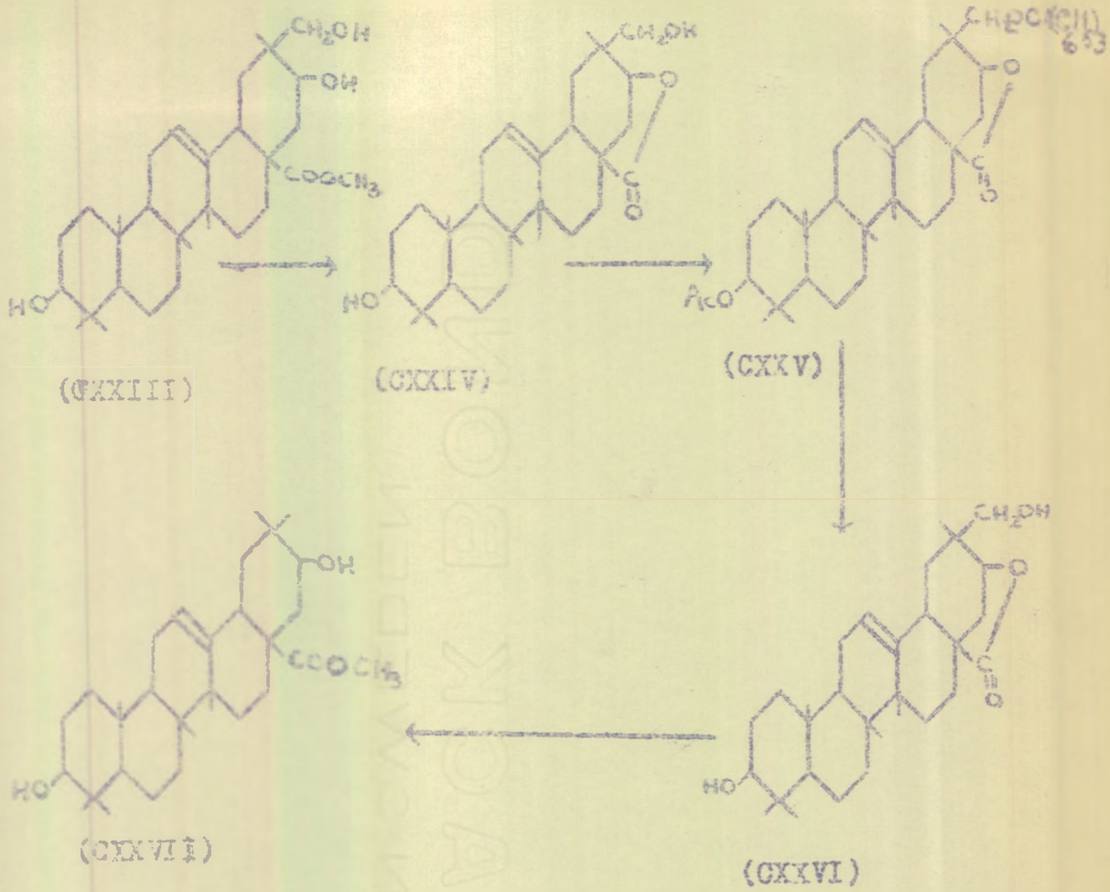


(CXXII)

The position of the third hydroxyl group at C-21 in trelleasegenic acid was concluded by relating it to machaerinic acid as indicated by the following sequence of reactions.

Methyl treleasegenate (CXXIII) on being refluxed with 5% sulphuric acid in water-dioxane gave a product (CXXIV) of the empirical formula $C_{30}H_{46}O_4$, which showed a strong indication of the presence of a five membered lactone ring in the infra red spectrum. It was tritylated at C-30 and acetylated to yield the acetoxy lactone 30-trityl ether (CXXV). Treatment with hydrogen chloride-chloroform resulted only in loss of the trityl group giving an intermediate (CXXVI) in which all the functional groups except the C-30 alcohol were protected. Chromium trioxide oxidation furnished the corresponding aldehyde lactone acetate, which upon Wolff-Kishner reduction followed by methylation (of the hydroxy acid formed by alkaline opening of the lactone) gave methyl machaerinate (CXXVII) further characterised as the 3β, 21β -diacetate.

The above described interconversions of treleasegenic acid with queretaroic acid and machaerinic acid provided rigorous proof for the structure and stereochemistry of the acid to be 21β, 30-dihydroxy oleanolic acid (CXI)



(CXXIII) (CXXIV) (CXXV)

(CXXVII) (CXXVI)

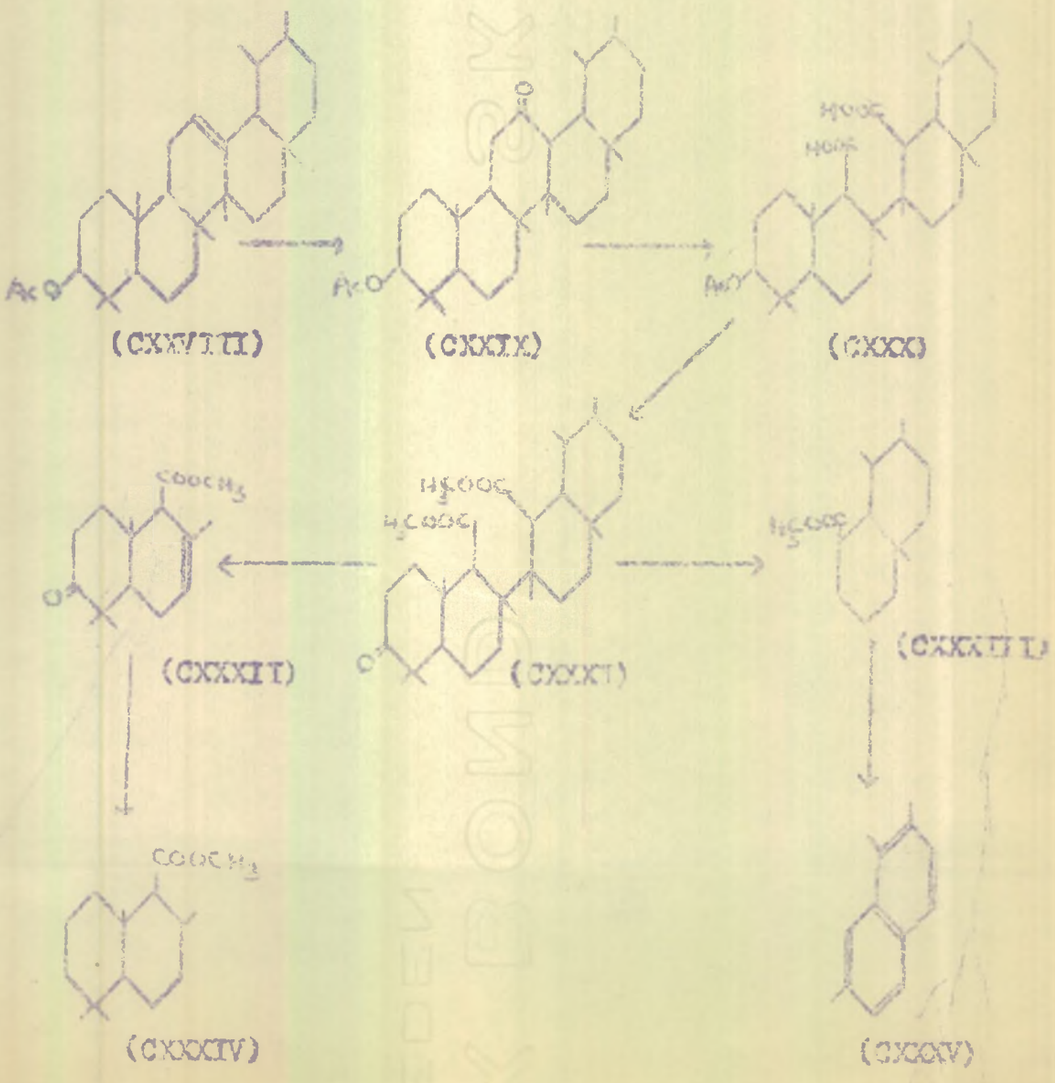
α-Amyrin group.

So far only a small number of compounds of this series have been classified. The close resemblance of α - and β-amyrins coupled with their identical selenium dehydrogenation products suggested that they were stereo-isomers. It was however, shown later by Ruzicka and collaborators[120] that they were structural isomers, differing only in the position of one methyl group in ring E.

The ethylenic linkage in this series is more sterically hindered (Cf. Table I) as the α-amyrin derivatives fail to give an epoxide or a bromolactone.

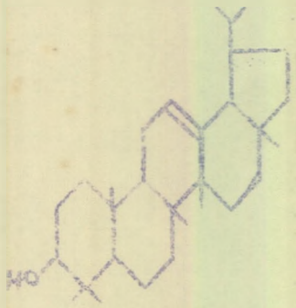
The molecular rotation studies of the two amyrins[121] indicate that in both the compounds the rings A and B are similarly constituted. This has been further confirmed by following series of reactions involving the fission of ring C.[122] α-amyrin acetate (CXXVIII) when reacted upon by ozone followed by treatment with acid or perhydrol and acetic acid yielded the ketone (CXXIX). Chromic acid oxidation of the ketone resulted in the formation of a dicarboxylic acid (CXXX). The methyl ester of the acid (CXXXI) on pyrolysis afforded a ketonic (CXXXII) and a non-ketonic methyl ester fraction (CXXXIII). The separation of the two fractions was carried out by Girard's reagent. The ketonic ester fraction (CXXXII) on Wolff-Kishner reduction gave an ester (CXXXIV) which was identical with the ester obtained from oleanolic acid (a β-amyrin derivative) by a similar treatment. Since the ketonic fraction arises from rings A and B, this provided a conclusive proof that the rings A and B in α- and β-amyrins were identical.

The non-ketonic fraction (CXXXIII) upon selenium dehydrogenation yielded sapotalene (1:2:7-trimethyl naphthalene) (CXXXV), whereas the parallel product from β-amyrin derivative was 2:7-dimethyl naphthalene. As these products arise from rings D and E, it is evident that α- and β-amyrins differ in the position of one methyl group in ring E.

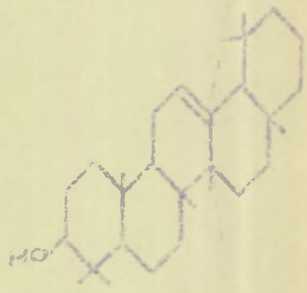


On the basis of the stability of cis-fusion of rings D and E and the conversion of Ursa-9(11):13(18)-dienyl acetate to Oleana-11:13(18)-dienyl acetate, Spring and collaborators[123] have proposed a new formula (CXXXVI) for α-amyrin in which the ring E is five membered and an isopropyl group is attached at C-19.

Meakins,[124] on the basis of the spectrographic studies of the tris-nor hydrocarbon prepared from the two amyrins and lupeol, showed the presence of two gem dimethyl groups in α-amyrin and thereby lent support to the structure suggested by Spring and collaborators and further suggested an alternative formula for α-amyrin (CXXXVII).



(CXXXVI)

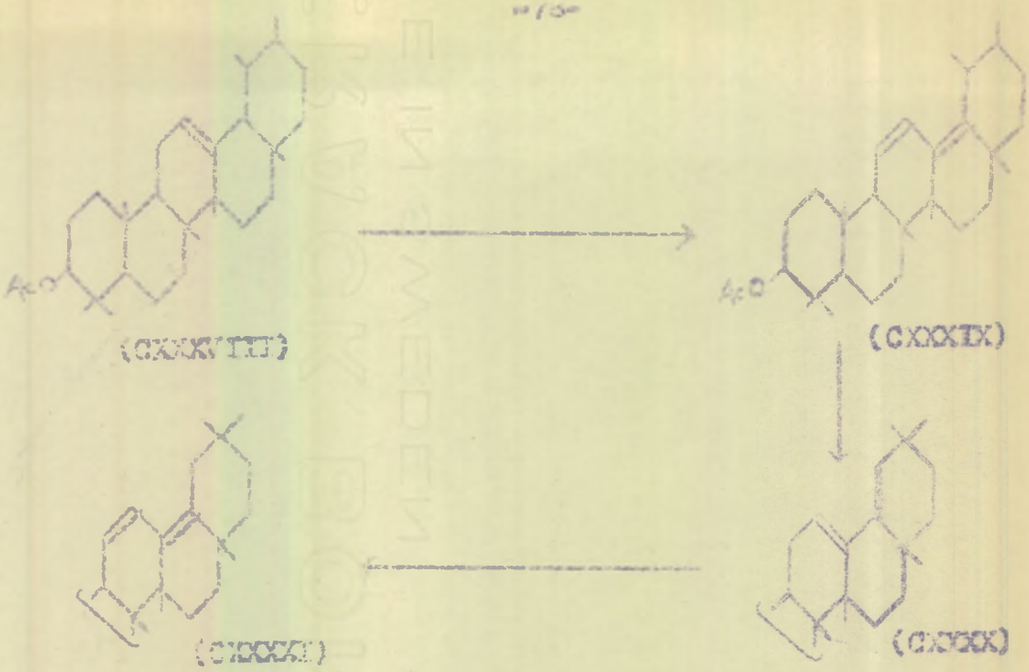


(CXXXII)

Cole and collaborators,[125] on the basis of the infra-red spectrographic studies of pentacyclic triterpenes especially in the region of the methyl bending absorption, do not agree with Meakins formula and are in favour of the one suggested by Ruzicka and coworkers[120] and later supported by Corey et al.[127].

A clearer picture of the structure and stereochemistry of α-amyrin was presented by Spring and coworkers[128] by the conversion of α-amyrin to a β-amyrin derivative of conclusively established structure and stereochemistry. α-amyrin acetate (CXXXVIII) on vigorous treatment with selenium dioxide gave (CXXXIX) in poor yield which on hydrochloric-acetic acid treatment gave the corresponding β-amyrin derivative (CXXXXI). Since in this transformation C-9, C-14 or C-17 are not involved, therefore the structure of α-amyrin may be expanded in the knowledge of the stereochemistry of β-amyrin.

As both α- and β-amyrins on selenium dioxide oxidation give the corresponding isodienonyl structure, the hydrogen at C-18 in α-amyrin may be β- as in β-amyrin.



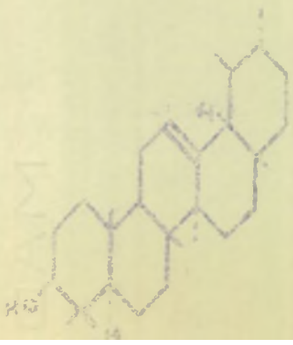
(CXXXVIII) (CXXXIX)

(CXXXXI) (CXXXX)

Optical studies of Corey and Ursprung[127] of the lactones of oleanolic acid and ursolic acid also prove that the configuration at C-17 and C-18 is similar in ursane and oleanane series.

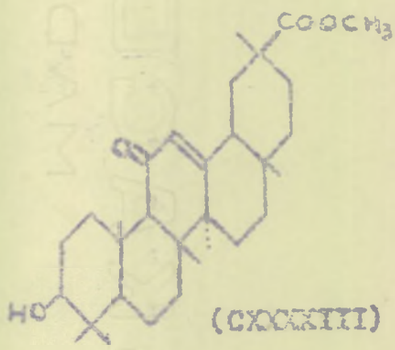
A further support in favour of the old structure of α-amyrin was available by the study of n.m.r. spectra[128] of α-amyrin.

On the basis of the above evidences the structure of α-amyrin may be written as follows:



(CXXXXII)

Further the above structure of α-amyrin has been confirmed by synthesising its acetate from methyl glycyrrhetate,[129] (CXXXXIII), which is a β-amyrin derivative.



(CXXXXIII)

Lupeol group

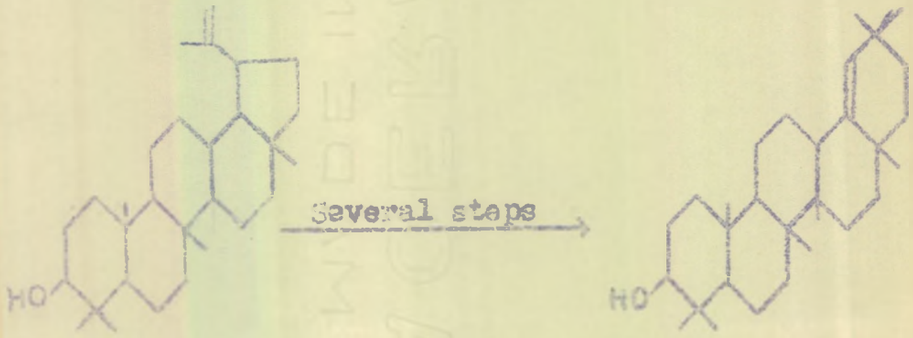
This group is comparatively smaller than the two amyrin groups (Table IV). Lupeol is the parent compound of this series. It was first isolated by Schulze and Steiger in 1889 from the seeds of Lupinus albus.[130]

Unlike the amyrins, the double bond in lupeol is easily hydrogenated[131] in presence of a platinum catalyst giving a saturated alcohol. Selenium dehydrogenation of lupeol yielded all the characteristic naphthalene derivatives, believed to arise from rings A and B, but failed to give 2:7-dimethyl naphthalene and 1:2:7-trimethyl naphthalene, which are considered to be the derivatives of the rings D and E of the amyrins. Picene and its hydroge

derivatives also could not be obtained, indicating a different pattern of the lupeol molecule.

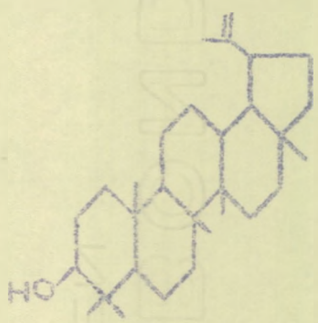
The easy saturation of the double bond in lupeol coupled with the formation of formaldehyde[132] as a result of ozonolysis suggested that the double bond was exocyclic in the form of a vinylidine group confirmed by the presence of a characteristic infra red band at 883 Cm^{-1} [133]. This also indicated that lupeol differed from the amyrins in the structure of rings D or E.

A controversy about the structure of lupeol was finally solved when Halsall, Jones and Meakins[134] conclusively established the structure of lupeol by its conversion to germanicol (3-hydroxy Δ^{18-19} oleanone) a β-amyrin derivative.(CXXXI) ----→ (CXXXV).



(CXXXIV) (CXXXV)

Now the structure of lupeol, $C_{30}H_{50}O$ compound which is finally accepted is as given below(CXXXVI).



(CXXXVI)

Betulinic acid, a well known member of this series, obtained from Cornus florida[135] has been shown to be 3 β -hydroxy lupene 28-oic acid.

Friedelin Group.

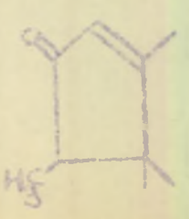
The wax cerin isolated from the cork by Chevreul[136] was later separated into two crystalline products friedelin and cerin.[137]

Drake and collaborators[138-140] carried out a thorough investigation of friedelin and showed it to be a saturated ketone (no yellow colour with tetranitromethane) with formula $C_{30}H_{50}O$. Reduction of the ketone with sodium and amyl alcohol yielded a secondary alcohol friedelanol $C_{30}H_{52}O$, while the Clemmenson reduction afforded

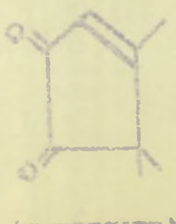
the saturated hydrocarbon friedelane $C_{30}H_{52}$. The carbon skeleton of the ketone was shown to be a reduced picene ring as selenium dehydrogenation gave all the characteristic naphthalene and picene derivatives which are obtained in the case of amyrins.[139] By a series of chemical reactions Ruzicka[141,142] and collaborators have provided evidence for the presence of -CH-CH-CO-CH_2-CH_2 group and thereby excluded the possibility of friedelin as being a member of amyrin series.

Later Spring[143] and Ourisson[144] and their collaborators showed that the nor-friedelenedione considered to be a C_{29} compound, had infact only 28 carbon atoms and should be designated as bis norfriedelenediene with the structure (CXXXVII). This therefore, implies structure (CXXXVIII) for norfriedelenone and its conversion to the diketone (CXXXVII) involves the loss of a methyl group.

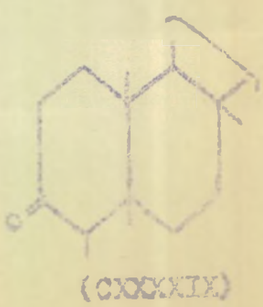
Swiss workers[141,142] in a series of reactions deduced the structure of ring B and the partial structure of friedelin[142,143] could be written as (CXXXIX).



(CXXXVIII)

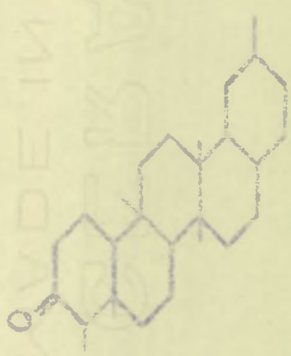


(CXXXVII)



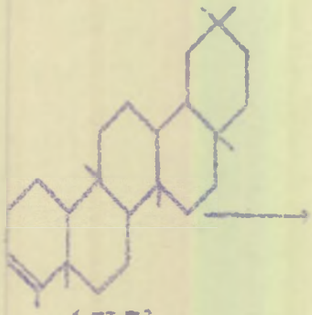
(CXXXIX)

Now the partial structure was fitted into the 1,8-dimethyl picene nucleus, which consists of 24 carbon atoms. Out of the six remaining carbon atoms which are present as methyl groups two can be located at C-4 and C-5. The other two methyl groups can be placed at C-13 and C-14 inorder to account for the formation of 1:2:7-trimethylnaphthalene. This allows the structure of friedelin to be as given below (CL).

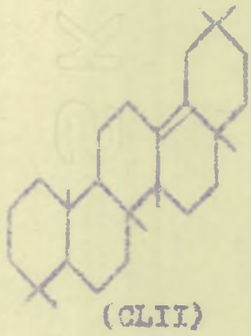


(CL)

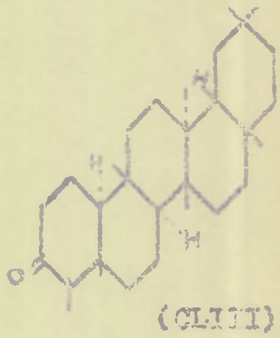
Recently a brilliant series of reactions[143-146] consisting in the conversion of friedel-3-one (CLI) to olean-13(18)-one (CLII) under strongly acidic conditions, requiring the migration of four methyl groups and two hydrogen atoms in an extra ordinary manner, has shown that friedelin has the following structure (CLIII).



(CLI)

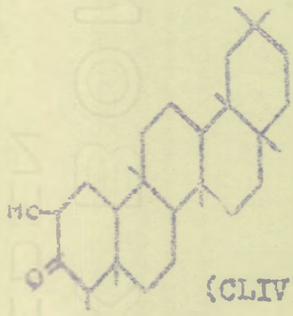


(CLII)



(CLIII)

On the basis of the structure of friedelin(CLIII)the structure of cerine can be written as friedeline 2β -ol (CLIV).



(CLIV)

Physical Methods.

Recently the application of physical methods in the elucidation of the structure of natural products has become very prevalent, and no doubt these methods have proved of immense value in solving various structural problems in this field.

Ultra violet spectroscopy

The Ultra-violet spectroscopy finds its main use in the terpene chemistry for the detection of conjugation.

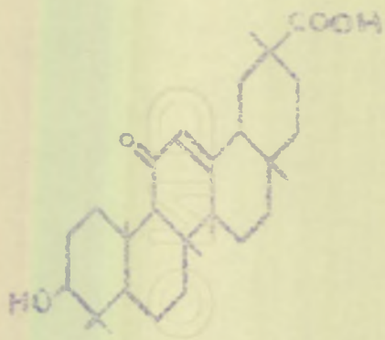
Henri[147], while working with mesityl oxide observed a strong band at236 mμ ($\log\epsilon$ = 4.1)as well as a weak band at 315 mμ ($\log\epsilon$ = 1.9). These two bands are now accepted as characteristic in general outline of all $\alpha\beta$-unsaturated ketones. Scheibe[148], Henschick[149] and their collaborators further extended these investigations and indicated that in general the presence of an $\alpha\beta$-unsaturated carbonyl system in a given compound could be correlated with intense selective absorption in the region 220-250 mμ.

Unsaturated esters, lactones and acids, can usually be recognised by their absorption maximum in the 220 mμ region and slow fall-off in intensity. A further application of the ultra violet spectroscopy includes

the $\alpha\beta$-diketone systems.

For the prediction of the high intensity bands of the system such as conjugated dienes and trienes, conjugated ketones, etc., the emperical rules have been proposed by Woodward[150,151] and modified by Fieser.[152]

In case of glycerrhetic acid[153] a consideration of the absorption maximum in the ultraviolet at 250 mμ log ∈ 4.1 has suggested that the acid is an $\alpha\beta$-unsaturated ketohydroxy acid.(CLV).



(CLV)

The triple ultra violet maxima at 243, 251 and 260 mμ have been found to be characteristic of the typical $\Delta^{11,13}$ (18) dienes of the β-amyrin series, obtained by selenium dioxide oxidation of the compounds. Thus on this basis as mentioned earlier, the members of the

β -amyrin series have been distinguished from those of the members of α -amyrin and lupeol groups.

The ultra violet maxima of α,β -unsaturated ketones, and conjugated dienes for a number of compounds in the triterpene series have been discussed and reviewed by Heller.[154]

The Infrared Spectra

The appearance of the absorption bands in a particular region of the infrared spectra and the displacement of these bands due to environmental differences formed the basic principle of Infrared spectrographic studies. The applicability of such a method, therefore, depends largely on the availability of reliable dat in the light of which the observations in the study of new compounds could be interpreted. Various aspects of the infrared spectra of steroids have been summarised by many workers.[155-57]

The infrared spectra of triterpenes have got much resemblance with the spectra of the steroids, but since the environments at each substituent position in the two types of systems, i.e. triterpenes and steroids are not identical, it is desirable to make a separate study of

triterpenes. For instance, the ring C in pentacyclic triterpenes, which is fused to two six membered rings B and D, is quite different from that in the steroids, where the D ring is five membered. The region of C - H stretching and bending absorption may alter the shape of the spectra due to the presence of the large number of angular methyl groups. The presence of C-4 gem-dimethyl group may also influence the C-3 substituent.

It has already been pointed out that, the effects of triterpenes are not identical with those found in steroid spectra for similar positions in C_5 ketones in the series of steroids the C-2 and C-4 methylene groups absorb near 1420 cm^{-1}, while in the corresponding (3-oxo-triterpenes the C-2, methylene group absorbs near 1430 cm a C-11 methylene in 12roxo steroids absorbs at 1434 cm whereas the same group in 12-oxotriterpenes absorbs clos to 1420 cm^{-1}.

A summary of the carbonyl band positions for pentacyclic triterpenes is given in the following table V.[158].

T A B L E V

CCl_4 solution		Carbonyl type	$CHCl_3$ solution	
μ	Cm^{-1}		Cm^{-1}	μ
5.634-5.643 5.747	1775-1772 1740	γ lactone $\Delta^{23,24}$-trisnor-3-ketone (five membered)	1756-1751 -	5.695-5.711 -
5.757-5.774 5.774	1737-32 1732	acetate aldehyde	1725-1717 -	5.797-5.824 -
5.770-5.804	1733-1723	Methyl ester	1725-1714	5.797-5.834
5.794-5.804	1726-1723	Formate	1714	5.834
5.814-5.824	1720-1717	Benzoate	1708-1707	5.855-5.858
5.841-	1712	19-ketone (oleanene series)	-	-
5.841	1712	20ketone (30-norlupane series)	-	-
5.851-5.862	1709-1706	3-ketone	1700-1698	5.882-5.889
5.858-5.862	1707-1706	11-ketone	1703	5.872
5.872-5.889	1703-1698	12-ketone	1696-1692	5.896-5.910
5.731-5.741	1745-1742	carboxylic acid	1714	5.834
5.896-5.928	1696-1687			
5.896	1696	conjugated aldehyde.	1690	5.917
5.851	1709	$\Delta^{13(18)}$-12,19	-	-
5.910	1692	-diketone		

CCl_4 Solution		Carbonyl type	$CHCl_3$ solution	
μ	CM^{-1}		Cm^{-1}	μ
5.921	1689	$\Delta^{13(18)}$-19-ketone(oleanane series)	1684	5.938
6.006	1665	Δ^{12}-11-ketone	1682	6.083
6.013	1663	$\Delta^{12(18)}$-dinene 11-ketone	1649	6.064

The detection and location of ethylenic double bonds in pentacyclic triterpenes has been made by a study of the frequencies and peak intensities of infra-red bands in the region of C =C stretching, C-H stretching, and C-H bending absorption.

The following table (VI)[159] gives a summary of the frequencies characteristic of ethylenic double bonds in pentacyclic triterpenes.

TABLE VI

Position of unsaturation	C = C stretching	C-H stretching	C-H bending	Adjacent CH_2
	cm^{-1}			
12:13	1650-1667(15-20)	3023-3030(sh)	804(30), 818(25) 828(30)	1435-1436
2:3	1659-1661(20)	3000-3010(sh)	729	
18:19	1645-1661(8-10)	3028-3030(sh)	802, variable	-
9:11	1660	-	819(40)	1437
20:29	1640-1642(55-70)	3070-3072 (35-45)	883(125)	-
3:18	-	-	-	1435
17:18	-	-	-	1436-1437
Δ^{12-18} Diene	1630	-	808, 819, 834	
$\Delta^{9(11):12}$ Diene	1635	3037	variable	-
$\Delta^{11-13(18)}$ Diene	1621-1627	-	808(12), 816(10)	
Δ^{12}-C_{11}-ketone	1618-1626(70-80)	3023-3030(sh)	806, 820(?) 828	-
$\Delta^{13(18)}$-C_{12}:C19 Diketone	1622(70)	-	-	

Recently on the basis of the Infra red results, Cole[160] and collaborators have characterised the equatorial or axial nature of the hydroxyl group in triterpenic compounds. The following table (VII) gives a summary of band frequencies, which are characteristic of equatorial and axial hydroxyl and acetoxyl groups at C-3 in triterpenes.

TABLE - VII

cm^{-1}

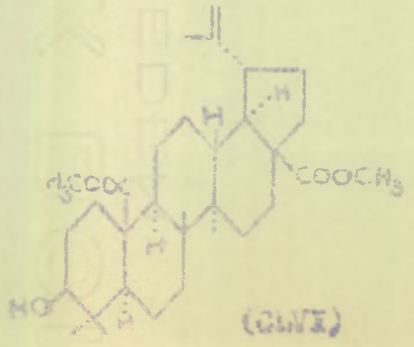
Hydroxyl groups			Acetoxyl groups
	CCl_4 solution	CS_2 solution	CS_2 solution
Equatorial	3629-3630	1013-1021, 1040-1045	1023-1025
Axial	3637-3639	1063-1068	1174-1188, 1038-1040

More recently[161] the infra red frequencies and intensities of hydroxyl absorption bands in triterpenes and similar compounds have been published. The following table gives the characteristic frequencies.

TABLE - VIII

Primary hydroxyl groups	3640-3641 Cm^{-1}
Axial secondary hydroxyl groups.	3635-3638 Cm^{-1}
Equatorial secondary	3628-3630 Cm^{-1} at C-3
	3623-3625 Cm^{-1} at C-11
Tertiary axial hydroxyl group	3617-3619 Cm^{-1}
Tertiary equatorial hydroxyl group	3613 Cm^{-1}

As a result of infrared spectroscopic studies, it might be possible to make a distinction between tertiary equatorial (3613 Cm^{-1}) and axial (3617 Cm^{-1}) hydroxyl groups. On this basis the band at 3629 Cm^{-1} (CCl_4) in methyl melaleucate[162] (CLVI) has been assigned as equatorial secondary, while its 3-epimer obtained by oxidation to the ketone and subsequent reduction (Meerwein Ponndorf) absorbs at 3636 Cm^{-1} as axial secondary.



(CLVI)

Molecular Rotation

The technique of molecular rotation differences applied very early in the field of carbohydrates, has found extensive application recently in the field of triterpenes and steroids. A study of the data on the molecular rotatory properties of all the known triterpenic and steroidal compounds led Jones and Barton[163-165] to conclude that the molecular rotations of triterpenic carboxylic acids and their esters are practically identical where molecular rotation M_D is given by the equation.

$$M_D = \frac{[\alpha]_D \times \text{Molecular Wt.}}{100}$$

The following table[163] (IX) gives the molecular rotations of some of the well known acids and their methyl esters belonging to the class of triterpenes. Where Δ is the molecular rotation difference between the acids and their esters.

TABLE - IX

Substance	10^{-2} M[M] Acid	Ester	$10^{-2}\Delta$
Ursolic acid	+ 319°	+ 324°	+ 5°
Hederagenin	+ 359°	+ 355°	- 4°
Betulonic acid	+ 141°	+ 145°	+ 4°

Barton and Jones[163] further noted that each of the three main groups of triterpenes ∝-amyrin, β-amyrin and lupeol showed very characteristic molecular rotation differences between the genin and its C-3 derivatives, such as acetate (Δ_1), benzoate(Δ_2)and ketone(Δ_3).

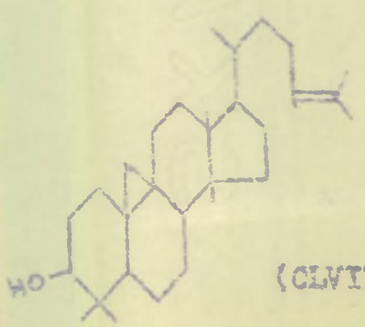
TABLE[166] X

Series	Δ_1	Δ_2	Δ_3
∝-amyrin (Urs-12-en-3 β-ol)	+ 6	+ 140	+ 110
β-amyrin(Olean-12-en-3 β ol)	+ 6	+ 150	+ 60
Lupeol(Lup-20(29)-en-3 β ol)	+70	+ 200	+140

A study of the above results indicates that this data can be used to distinguish between amyrins and lupeol group. (see major difference in Δ_1 and Δ_2).

A number of erroneous conclusions have been corrected on the basis of the molecular rotation evidence and the structures of the well known compounds have been further confirmed. Thus Barton and Jones[163] were able to suggest that α-viscol and gratiolone were identical with β-amyrin and betulinic acid respectively. This was subsequently confirmed by a comparison of the derivatives of gratiolone and betulinic acid.[167]

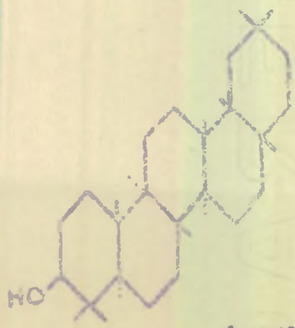
A well defined and characteristic difference has been observed between the molecular rotations of steroids and triterpenes[164], which can be used for assigning the two substances to their respective classes. Cycloartenol (CLVII) previously considered to be a steroid has been shown to be a triterpene derivative because the changes in molecular rotation, on acetylation and benzoylation of the compound, are comparable with those recorded for triterpenic compounds.[168]



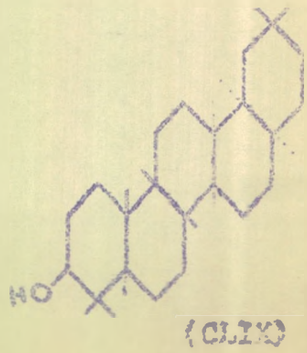
(CLVII)

Recently Klyne[169-71] has extended the method, for the stereochemical study, to this series, wherein the stereochemistry of one group of polycyclic compounds has been correlated with that of another. The rule of shift has been applied in a general form to the molecular optical rotation of polycyclic compounds. The basis of the principle is that the terminal ring units of the same type make contributions to molecular rotations, which are almost independent of the rest of the molecule, provided the penultimate ring is a saturated unsubstituted cyclohexane ring.

Klyne has further utilised these molecular rotation differences for the determination of the stereochemistry of the terminal rings of the polycyclic compounds such as triterpenes. On the basis of the molecular rotation arguments he has favoured the formula (CLVIII) for β-amyrin in preference to (CLIX).



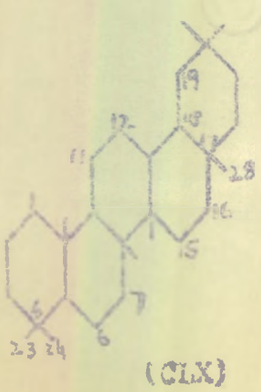
(CLVIII)



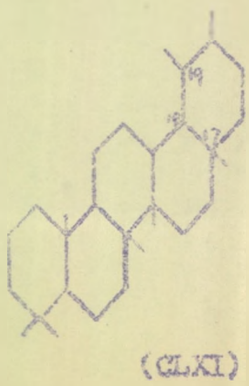
(CLIX)

Klyne and Stokes[171] elaborating the work of Stokes and Bergmann[172] have stated some general rules regarding the rotation contribution of hydroxyl, acetoxyl and benzoyloxyl groups in alicyclic compounds. The principles may be used to correlate the stereochemistry of triterpenes with that of steroids.

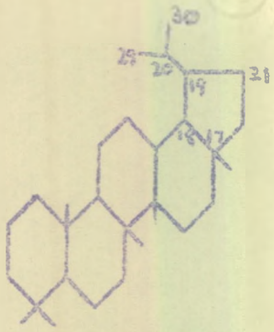
In the case of pentacyclic triterpenes of oleanane (CLX), ursane (CLXI) and lupane (CLXII) series, the position 7,11 and 16 are similar to positions 4,7 and 11 in steroids (CLXIII). Therefore Δ OH, Δ OAc, Δ OBz, Δ_1 and Δ_2 should be negative for α-substituents and positive for β-substituents. The positions 1,6, and 12 or 15 resemble positions 1,6 and 12, in the steroid series the Δ values should here be positive for α-substituents and negative for β-substituents. The above predicted signs have been found in good agreement with the experimental values.



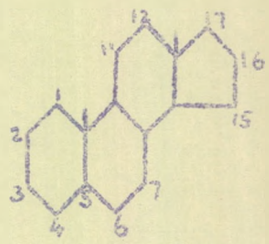
(CLX)



(CLXI)



(CLXIII)

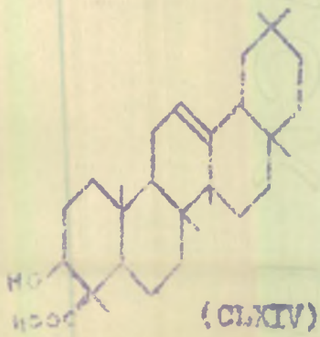


(CLXIII)

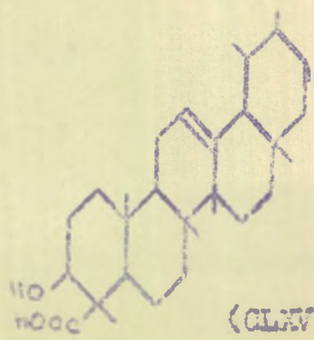
On the basis of the molecular rotation evidence Klyne and Stokes[171] have supported the 3α -configuration of the hydroxyl group in α -boswellic acid (CLXIV) for which the △ values for the methyl esters are

$$\triangle OH = -80 \qquad \triangle OAc = -268 \qquad \triangle_{5} = -188$$

In the case of β -Boswellic acid (CLXV) as well, the △1 are all large and negative indicating a 3 α -hydroxyl group.



(CLXIV)



(CLXV)

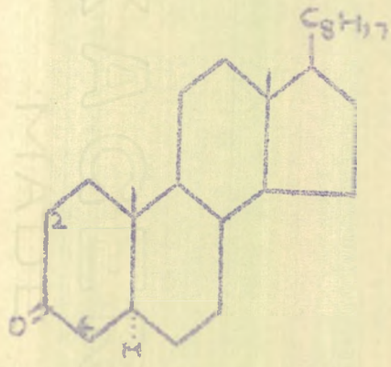
Optical Rotatory Dispersion

Optical rotatory dispersion, the change in optical rotation with the wavelength, although observed by Biot[173] in 1817, has only recently been applied by organic chemists in the solution of some structural and stereochemical problems. The introduction of Rudolph's spectropolarimeter as a very convenient and quick method for these measurements enabled Djerassi and collaborators to explore such a possibility on a large scale, and have met with success within the last few years. No doubt, optical rotatory dispersion, another physical method has a promising future in the field of organic chemistry.

The optical rotations can now be measured over a range of 250-700 mμ, and the curves so obtained provide means for some information regarding the structural and stereochemical features of the compound under investigation.

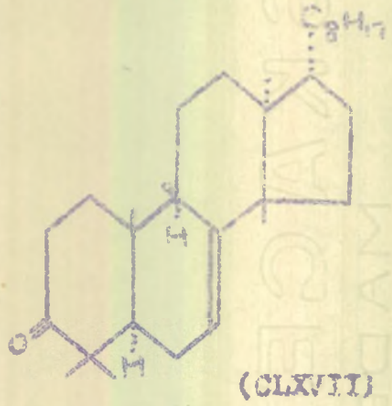
The use of rotatory dispersion curves in solving the structural problems in the field of steroid chemistry is more than five years old. In his first paper on rotatory dispersion, Djerassi[174] had pointed out that if the rotatory dispersion studies proved fruitful in the steroid series, they should also be of an equal importance in the triterpenic chemistry.

Since, the tetracyclic triterpenes[175] can be considered to be the trimethyl steroids, the change in cotton effect curve by methylation of 3-keto-5 α-steroid cholestan-3 one (CLXVI) was studied. It was found[176] that neither 2 α-, 2 β-, 4 α-, and 4 β-methyl cholestan -3-one nor a 2,2-dimethyl grouping affected the sign of the cotton effect curve. However, by the introduction of two methyl groups in position 4 as in the case of 4,4-dimethyl-3-keto-5 α-steroid an inverted cotton effect curve was obtained as compared to that of the unmethylated parent ketone. This was an important observation, since the 3-keto-4,4-dimethyl grouping is the structural unit of nearly all the tetracyclic triterpenes.

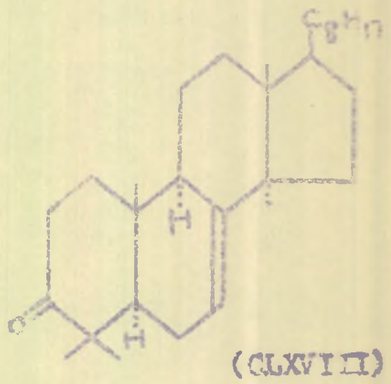


(CLXVI)

A resemblance in the cotton effect curve of dihydrobutyrospermone (CLXVII) with that of Δ^{7}-lanosten-3-one (CLXVIII) offers an excellent evidence in favour of 9α-orientation of dihydrobutyrospermone, a long debated point.[177-78]



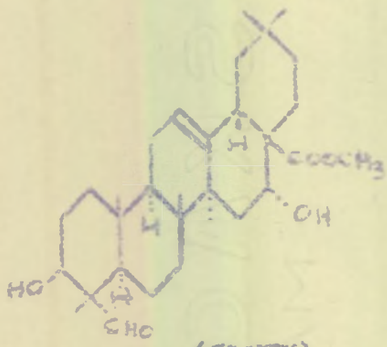
(CLXVII)



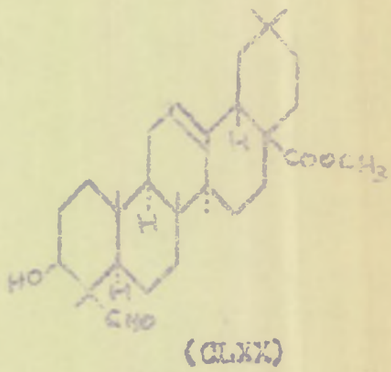
(CLXVIII)

The application of the optical rotatory dispersion curves in the pentacyclic triterpenes can be demonstrated by considering the case of quillaic acid (CLXIX) and gypsogenin (CLXX). The stereochemical identity of ring A in both these compounds by chemical transformations[179] was deduced with great difficulty, but its confirmation with the help of similar rotatory dispersion curves[180] utilizing the presence of aldehydic group was a simple affair. The curves of the above two compounds differ

considerably with the rotatory dispersion curve of gummosogenin which has got an aldehydic group attached at C-17.

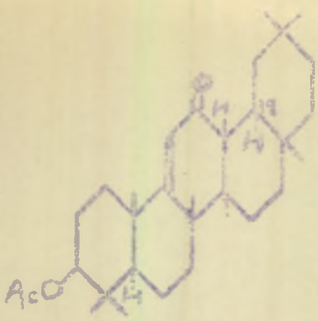


(CLXIX)

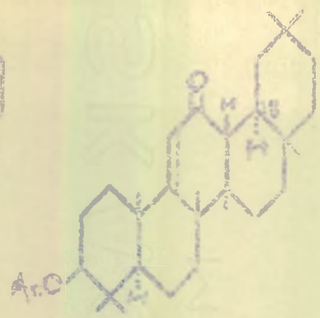


(CLXX)

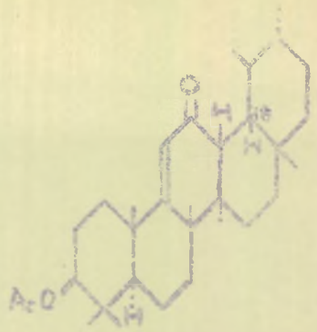
The rotatory dispersion curve of $\Delta^{9(11)}$- oleanen 3 β -ol-12-one acetate (CLXXI) differs considerably from that of the $\Delta^{9(11)}$-18 α -oleanen-3 β -ol-12-one acetate (CLXXII). The two compounds are the stereoisomers with the only difference in the orientation of the hydrogen atom at C-18. The dispersion curve of the corresponding α -amyrin derivative $\Delta^{9(11)}$-ursen-3 β ol-12 one acetate (CLXXIII) resembles well that of (CLXXI), a 18 β -isomer, therefore a correct β -orientation to 18-hydrogen has been assigned in this compound.



(CLXXI)

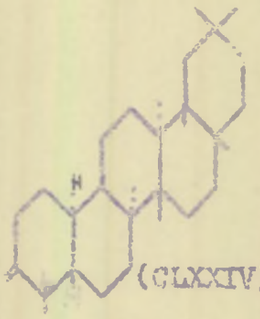


(CLXXII)

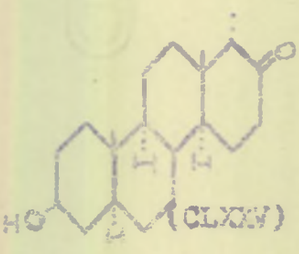


(CLXXIII)

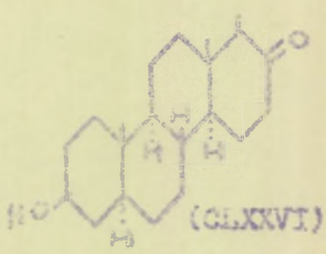
A comparison of the dispersion curves of friedelin (CLXXIV); 17 α(α -methyl D -homoandrostan-3 β -ol -17-one and 17 α β -methyl -D -homoandrostan-3 β ol -17-one showed that the curve of friedelin resembles closely that of the equatorially oriented 17α β-methyl ketone (CLXXVI). As the stereochemistry of rings A and B of friedelin is identical with that of the rings C and D in (CLXXV) and (CLXXVI), it was concluded that the C -4 methyl group of friedelin had the equatorial orientation, which is an established fact.[145]



(CLXXIV)



(CLXXV)

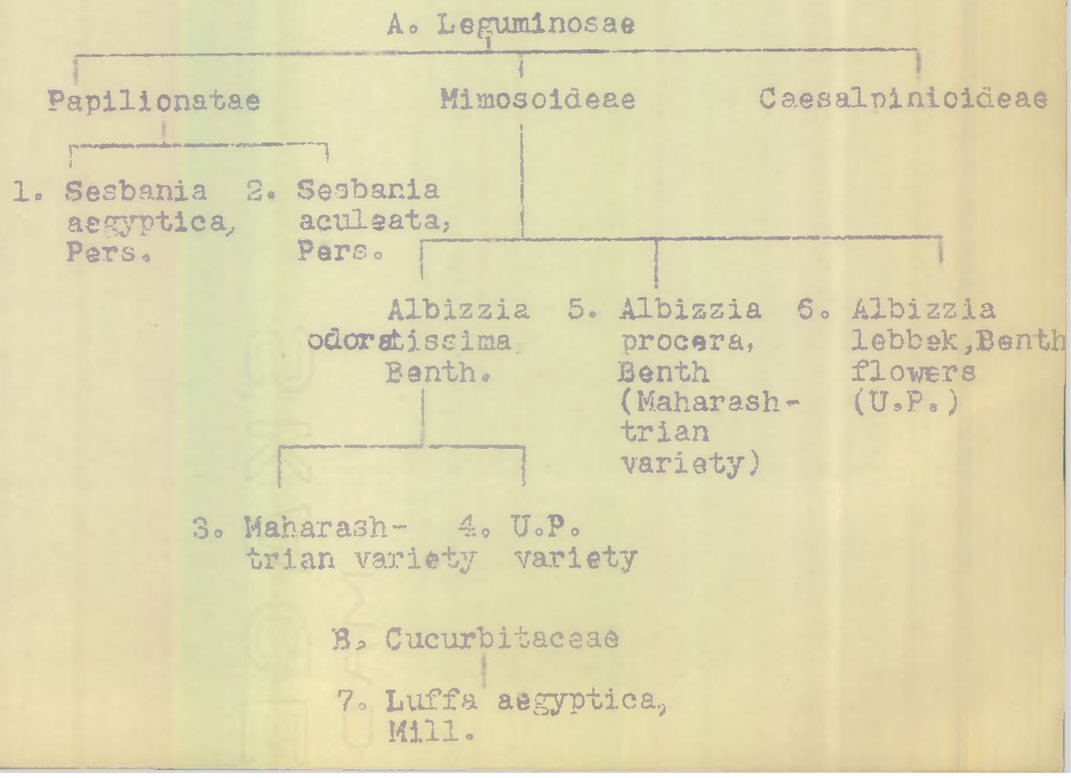


(CLXXVI)

PRESENT WORK

Present Work

The saponins are one of the constituents found in various parts of the plants and have been the subject matter of detailed study widely throughout the world. India is very rich in the plant wealth and therefore this type of work on sapogenins has been taken up with a view to isolate some new sapogenins, to establish their constitution and to study ultimately their biogenetic relationship. The present work is a part of this study spread over only on the families of Leguminosae and Cucurbitaceae, concentrating chiefly on one family i.e. Leguminosae.



A. Family Leguminosae

1. The study of the seeds of Sesbania aegyptica,Pers.

Sesbania aegyptica,Pers. commonly known as "Ravasin", belongs to the family leguminosae, sub-family, papilionatae, and is a weedy tree like herb, 6-10 feet high, growing wildly throughout the plains of India. It is used as a hedge plant due to its rapid growth. The seeds have been reported to reduce enlargement of spleen and as an ointment to eruptions.[181]

A number of the members of the family leguminosae have earlier been reported to contain steroidal and triterpenic saponins and sapogenins.[182-185] As no mention of the work on the saponin and sapogenin from this plant has been found in literature, the study of the saponin and sapogenin from it was taken up. One paper has been published on this subject in the Journal of the American Pharmaceutical Association, Scientific edition,1959,48, 466-68 and is attached at the end of the thesis.

2. The study of the seeds of Sesbania aculeata,Pers.

In the course of our work on the saponins and sapogenins from Sesbania aegyptica,Pers. described above, it was noted that the only other species of Sesbania available easily is Sesbania aculeata,Pers. As no work is reported in literature on this variety as well, it

was thought desirable to study these seeds for their saponin and sapogenin contents.

Sesbania aculeata Pers. commonly known as "Dhaincha", is a member of the family leguminosae, sub-family papilionatae and grows from the West Himalayas throughout the plains of India. It is a soft wooled, erect, herbaceous annual plant reaching several feet in height. Cattle eat this plant before flowering. Sheep and goats are especially fond of its leaves. The plant is used as a green manure in certain parts of India.[131-86]

The supply of the seeds of Sesbania aculeata, Pers. was obtained from M/S. Jawahar and Sons, Saharanpur (U.P.). A quantity of the well powdered seeds was defatted with light petroleum ether (40-60°) in a soxhlet apparatus. The defatted seed powder was exhausted with ethanol. The recovery of the solvent left an oily residue which was extracted with petroleum ether, ether, carbontetrachloride and acetone in order to remove the soluble impurities. This left a brown semi-solid residue. The residue was dissolved in a small quantity of alcohol, filtered and repeatedly precipitated by addition to a large volume of ether and filtered on a Buchner funnel. Finally, the precipitation was made by dissolution of the substance in a small quantity of alcohol and adding it to a large volume of acetone, which gave a light cream coloured hygroscopic powder giving all the tests for saponins[22].

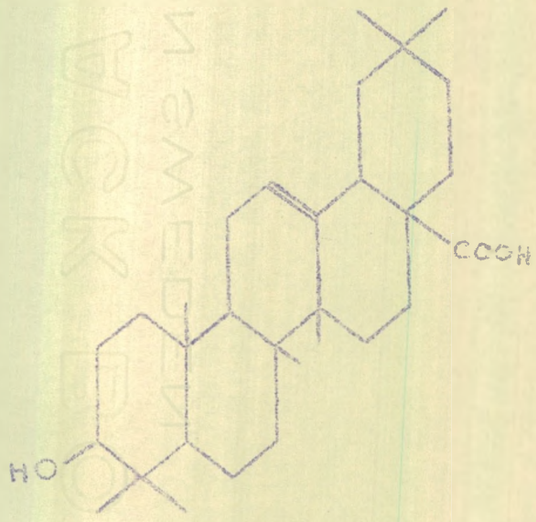
The saponin thus obtained was dissolved in a large amount of water and hydrolysed with sulphuric acid by heating the solution first on a boiling water-bath and thereafter by refluxing it. At the end when the hydrolysis was complete, the contents were cooled and filtered. A brown coloured precipitate obtained was washed several times with water in order to remove any acid left out. It was separated into acid and neutral fractions by sodium salt formation and extraction with ether. The ether on recovery left a residue, which was acetylated in the usual manner with acetic anhydride in pyridine. On crystallisation from methyl alcohol it gave the neutral genin acetate as colourless crystals m.p.185-90°C. The alkaline solution left after ether extraction was decomposed by hydrochloric acid which precipitated the acid genin. It was filtered washed with water and dried. On acetylation with acetic anhydride and pyridine in the cold it gave the acid genin acetate m.p.262-64°. The acetate gave a positive Liebermann-Burchard reaction and all the colour tests of triterpenes. It gave a yellow colour with tetranitromethane showing the presence of atleast one carbon-carbon double bond.

Methyl ester of acid genin acetate:

The acetate on treatment with an ethereal solution of diazomethane gave acetyl methyl ester m.p.220-21°, which also showed unsaturation with tetranitromethane.

As sesbania aegyptica gave oleanolic acid and the melting points of this genin derivatives are nearing that of the derivatives of oleanolic acid, they were compared with them. The acetyl methyl ester m.p.220-21° and the acid genin acetate m.p.262-64° when mixed melted with the corresponding authentic samples of oleanolic acid derivatives did not depress the melting points, indicating the present genin to be oleanolic acid.

The infra-red spectra of the acetate m.p.262-64° and the authentic sample of oleanolic acid acetate were superimposable, (Fig.I) thus establishing the identity of the acid genin as oleanolic acid (CLXXVII).



(CLXXVII)

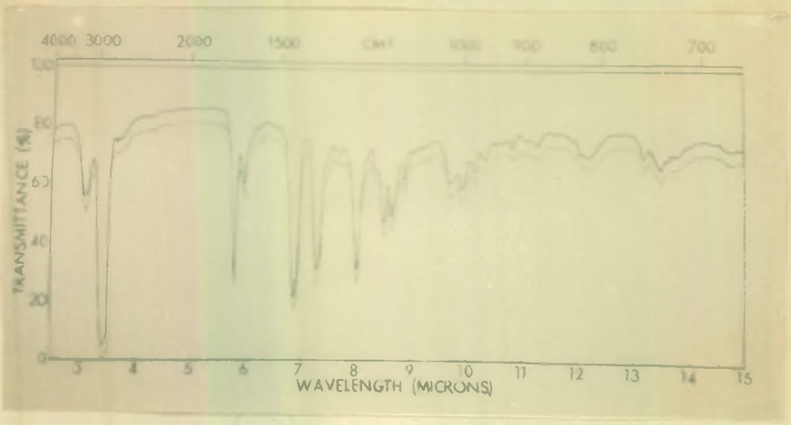
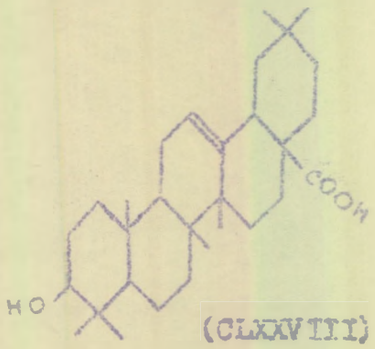


Fig.1

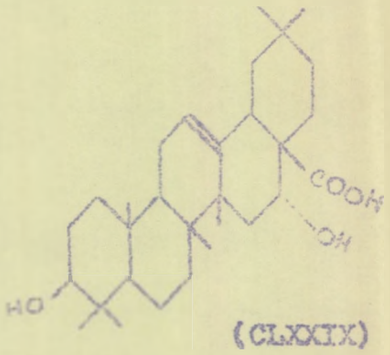
3. The study of the seeds of Albizzia odoratissima,Benth. from Maharashtra.

Albizzia odoratissima,Benth.commonly known as "Kala Siris" is a large erect tree of 25-30 feet height and is found throughout India. Its fruits are 6 to 8 inches in length resembling those of Albizzia lebbek and contain 8-10 seeds. The leaves are used as a remedy for cough and the bark in leprosy.[187.188] It belongs to the family Leguminosae and sub-family Mimosoideae. A review to the literature showed that no work has been done on any part of this plant except on the oil obtained from the seeds which have been studied for their fatty acid composition by Farooq and Siddiqui[189] in these laboratories.

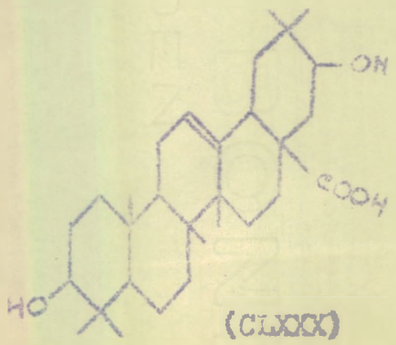
The other members of the family leguminoseae viz. Albizzia lebbek,[183] Albizzia procera,[184] Albizzia anthelmintica[182] have been found to contain triterpenic saponins. The seeds of Albizzia lebbek have been reported by Farooq, Varshney, Sannié and others[183,212] to contain a mixture of saponins which on hydrolysis yields oleanolic acid (CLXXVIII) and echinocystic acid (CLXXIX). The seeds of Albizzia procera, studied in these laboratories by Farooq, Varshney and Hasan[184] have been found to yield a new saponin proceranin which on hydrolysis yields machaerinic acid (CLXXX).



(CLXXVIII)



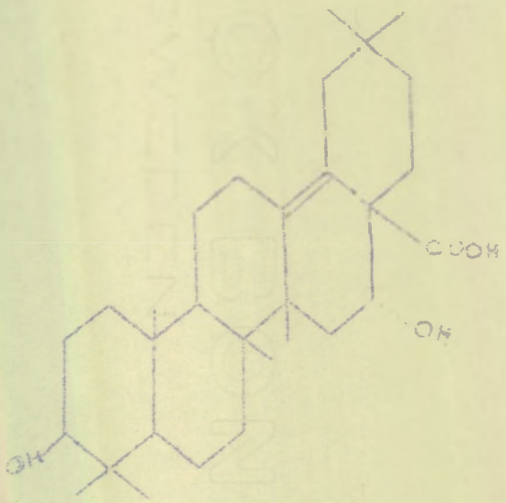
(CLXXIX)



(CLXXX)

In addition to these seeds of Indian origin the bark of Albizzia anthelmintica from Germany has also been studied by Tschesche and others[182] and found to contain musennin, a new saponin of echinocystic acid.

Quite recently Barua and Raman[190] studied the whole beans of Albizzia lebbek from Bengal and have reported the isolation of albigenic acid (CLXXXI) an isomer of echinocystic acid (CLXXIX) in addition to echinocystic acid (CLXXIX) and oleanolic acid (CLXXVIII).



(CLXXXI)

It is regretted that these authors have not studied the seeds and the beans separately but studied as a whole, and it is probable that this additional acid, albigenic acid (CLXXXI) which is an isomer of echinocystic acid (CLXXIX) may be present in the beans and not in the seeds.

Further it may be pointed out here that these authors have hydrolysed the saponin by utilising hydrochloric acid as a hydrolysing medium in comparison to sulphuric acid used by Varshney et al.[183] It is not rare that the hydrochloric acid treatment brings about an isomerisation in the molecule of triterpenes, viz. the isomerisation of olean-12-ene to olean-13(18)-ene, brought about by Spring and collaborators,[191] and the isomerisation of olean-12-en-3 one to olean-13(18)-en-3one reported by Ames and Jones.[192] Therefore, the presence of this acid (CLXXXI) can also be a result of isomerization brought about by hydrochloric acid in echinocystic acid (CLXXIX) which is already reported to be present. Although, Barua and Raman[190] have attempted to bring about the isomerisation in free echinocystic acid by treatment with hydrochloric acid with negative results, it may be noted here that an ordinary treatment with hydrochloric acid may not bring about any isomerization, but if the hydrolysis of the saponin is attemnted at the same time, the isomerization in the course of hydrolysis may be possible. There may be another reason that Albizzia lebbek from Bengal might be different and may have contained this acid which is not present in the seeds from Uttar Pradesh,[183] as it has been noted that the soil and climate have got a marked effect of this type on the plant constituents.

As no work on the saponin from the seeds of Albizzia odoratissima was reported in the literature, the seeds obtained from the Silviculturist, Maharashtra, Poona, were tested for the presence of saponin. The preliminary tests showed a considerable amount of saponin and therefore it was thought desirable to investigate these seeds for their saponin and sapogenin constituents.

A quantity of the finely powdered seeds was well defatted with light petroleum ether (40-60°), and then exhausted with ethanol. The recovery of the solvent left an oily residue which was successively treated with petroleum ether, ether, carbontetrachloride, chloroform and acetone. The residue left over was dissolved in ethyl alcohol and precipitated by addition to a large amount of ether. The operation was repeated several times, and finally the precipitation was made by addition to a large volume of dry acetone. This gave a light cream coloured hygroscopic powder. It caused sneezing, produced abundant foam on shaking with water and was toxic to fishes in low concentrations. It caused the haemolysis of the red blood corpuscles in very low concentrations.

The saponin was dissolved in a large amount of water and hydrolysed with sulphuric acid by heating the solution on a boiling water bath for one hour and thereafter refluxing it for another hour to complete the hydrolysis. A

light brown precipitate of the genin was obtained which was filtered, washed with water, free of sulphuric acid and dried.

The genin thus obtained was refluxed with alcoholic solution of potassium hydroxide for half an hour and then half of the solvent was recovered. The contents were diluted with sufficient quantity of water and extracted a number of times, with ether to remove any neutral genin, if present. On recovery of the ether no appreciable quantity of the neutral genin was obtained.

The ether extracted alkaline solution was acidified with hydrochloric acid, which precipitated the acid genin. The genin was filtered washed with water till the washings were neutral and dried. It was acetylated with pyridine and acetic anhydride in the cold. The contents were poured into ice-water. The precipitate obtained was filtered and washed free of pyridine and acetic acid. It was dried and crystallised from methanol containing a little chloroform It gave two types of crystals of the acetate, quite different from each other. They were separated by fractional crystallisation following a triangular scheme, and were obtained in two types of colourless needles in about 1:1 proportion, m.p. 260-65° and m.p. 228-34°.

(A) Study of the acetate m.p. 260-65: The analysis of the acetate m.p. 260-65° showed the formation of a di-

acetate corresponding to the formula $C_{34}H_{52}O_6$. The diacetate gave positive test for the double bond with tetranitromethane. The infra-red spectra of the acetate (Fig.2) showed the presence of an acetate band at 1257 cm^{-1}. The absence of a free OH group was evident by the absence of any sharp absorption in 2 μ region.

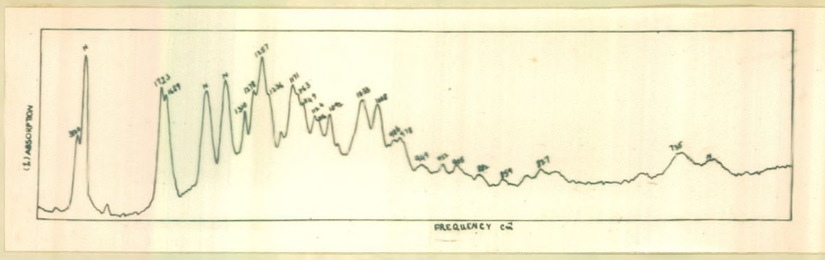


Fig.2

The acetate was deacetylated by refluxing with methyl alcoholic potassium hydroxide and decomposition of potassium salt by hydrochloric acid. It was filtered, washed free of acid, and dried. On crystallisation from methyl alcohol, it gave colourless crystals m.p.266-70°. The analysis of the genin led to the presence of two hydroxyl groups and one carboxyl group in a pentacyclic triterpenic compound. The infra-red spectra of the genin (Fig.3) is consistent with this formulation and resembles with the spectra of the triterpenic acids of β-amyrin

group. It showed the absorption between 11.8-12.4 μ indicative of the presence of a triply substituted ethylenic linkage (Cf. Barton et al,[89] King et al[107], Ruzicka et al[193]). The colour reactions of this sapogenin were positive for the triterpenes.

Fig.3

It gave a yellow colour with tetranitromethane confirming the presence of at least one carbon-carbon double bond which was also shown by the infra-red spectra. The acid being virtually insoluble in a solution of sodium bicarbonate, showed its weak acidic nature. The ease of the acetylation and saponification of all the two acetyl groups suggested that the two hydroxyl groups were either equatorially oriented secondary and/or primary alcoholic functions.

An ethereal solution of the genin on treatment with diazomethane gave on crystallisation from methyl alcohol a methyl ester m.p.224-25°, ~~(I.R.Spectra, Fig.4)~~, which readily detected and fixed up the carboxyl function. The diacetate on a similar treatment with diazomethane gave a diacetyl methyl ester m.p.279-80°. (I.R.Spectra, Fig.4) The analysis of the diacetyl methyl ester showed the genin to be a monocarboxylic acid. All these derivatives showed that the present genin has got two hydroxyl groups and one carboxyl group and belongs to the β -amyrin series of triterpenes.

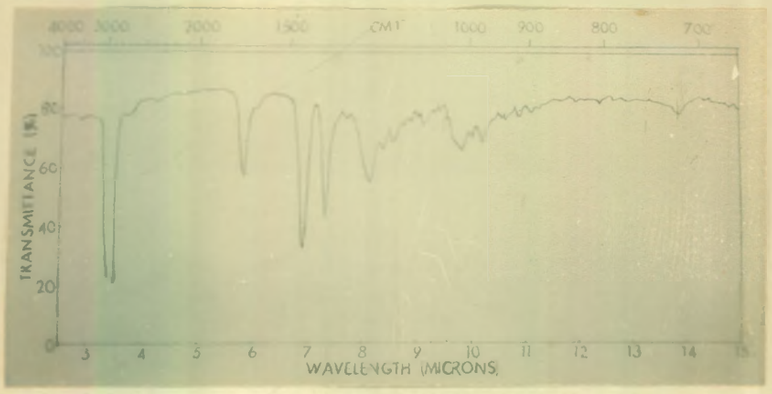
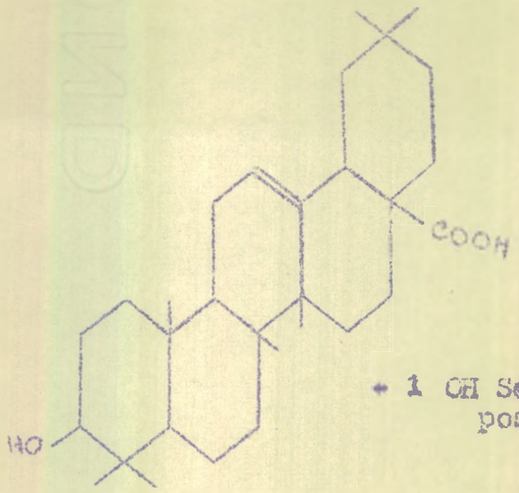


Fig.4

The formation of the bromolactone in very good yield has been utilised to fix up the position of the double bond and carboxylic acid, as well as to prove the relation with β -amyrin group. It is a well known fact

that the carboxyl groups at C-28 attached to C-17 in the compounds of β-amyrin series lactonise very easily with double bond, when reacted upon by bromine, to give the highly crystalline bromolactones in very good yield. (Cf. King et al [107,194], Jegar[87]). This also fixes up, in addition to the relation of β-amyrin group, the position of the double bond and carboxylic group (βγ or γδ).

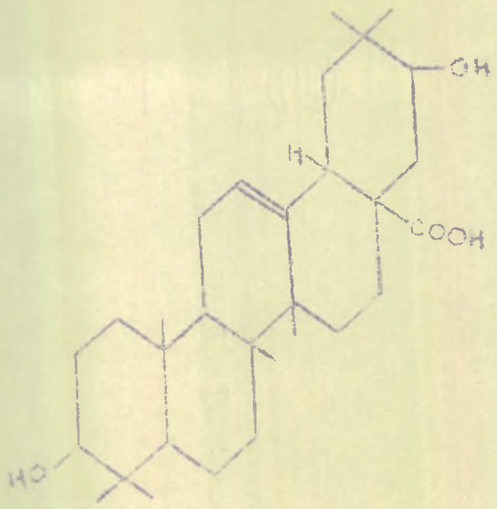
The action of bromine in acetic acid in presence of sodium acetate on the diacetate m.p.260-65° gave an acetyl bromolactone in about 95% yield m.p.288-90°. The bromolactone gave negative test for the presence of carbon carbon double bond with tetranitromethane showing the formation of a lactone between carboxyl group and the double bond. This formation of the bromolactone in very good yield gave further support to the olene-12-ene structure for this acid and also fixed up the position of the double bond between C-12 and C-13 and of the carboxyl group at position 28. Thus the following structure (CLXXXII) for the present genin was assigned.



(CLXXXII)

The comparison of the physical constants of the genin and all its d rivatives with all the known acids of the β -amyrin group containing two hydroxyl groups (Cf. table No.II p.45-49) indicated that the present acid can be identical with machaerinic acid (3 β , 21 β dihydroxy Δ^{12}-18 β oleanene-28-oic acid)(CLXXXIII).

The mixed melting points of all the derivatives and the genin with authentic samples of machaerinic acid and derivatives (Table No.XI) earlier obtained by Farooq, Varshney and Hasan[184] from Albizzia procera,Benth seeds of Madhya Pradesh origin, confirmed the present genin to be machaerinic acid (CLXXXIII).



(CLXXXIII)

Machaerinic acid.

Table XI

	Machaerinic acid	Present genin	Mixed melting point
Genin	256-58°	266-70°	266-70°
Acetate	258-60°	260-65°	260-64°
Methyl ester	224-25°	224-25°	223-25°
Ac methyl ester	278-80°	279-80°	279-80°
Ac Br-lactone	276-78°	283-90°	273-77°

The infra red spectra of the acetyl methyl ester and acetyl methyl machaerinate, which are superimposable (Fig.5) further confirmed its identity as machaerinic acid (CLXXXXII).

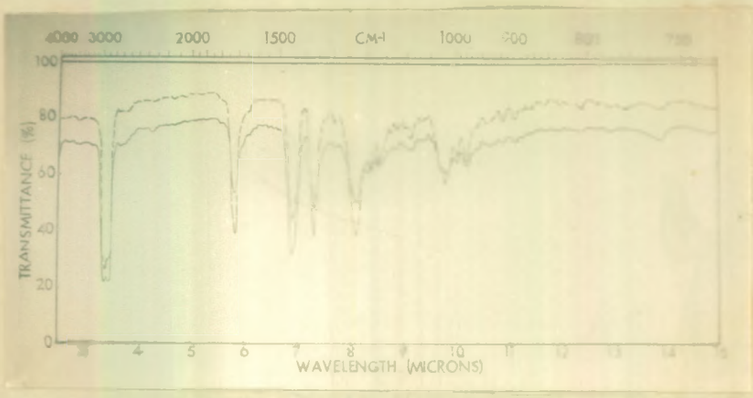


Fig.6

(B) Study of the acetate m.p.228-34.

Repeated attempts were made to purify this acetate by crystallisation from various solvents with no better results. It was therefore desirable to transform it into acetate methyl ester, inorder to purify it further, for getting better melting point. In this attempt, the acetate was reacted with a solution of diazomethane for 24 hours and the product obtained was crystallised from methanol as colourless needles m.p.232-34°. The form of the crystals was found to be the same as that of the

starting material, hence the mixed m.p. was taken with the acetate, when no depression was noted. This showed that the esterification had not taken place and to confirm it, this was studied by infra-red spectroscopy. The infra red spectra of the acetate and the product after esterification were identical proving the contention that no esterification had taken place. The study of the infra-red spectra showed a lactone band similar to the one in acacic acid acetate[195]. A comparison of the above spectra with the spectra of acacic acid acetate confirmed it to be identical with Acacic acid acetate (Fig.6). This proved beyond any doubt that the present acid is acacic acid. The $LiAlH_4$ reduction of the acid genin acetate gave a product m.p.295-98° identical with the tetrol obtained by Farooq, Varshney and Naim[196] from acacic acid on similar treatment.

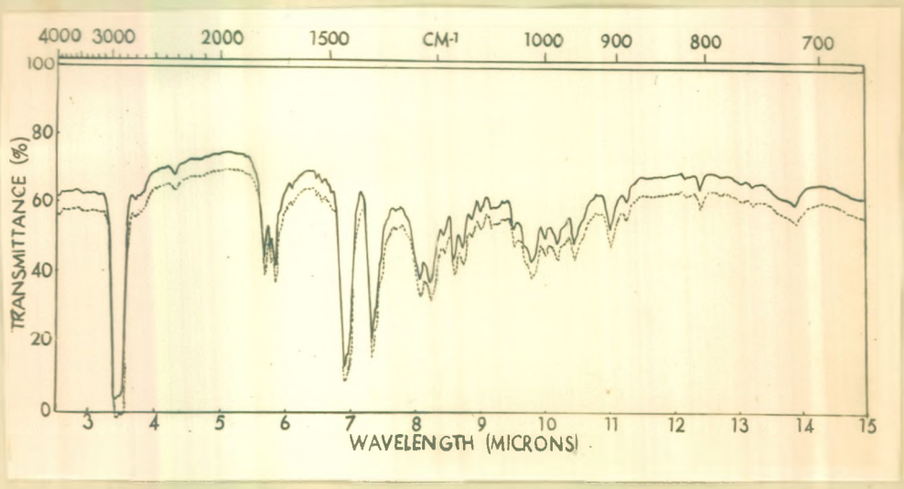
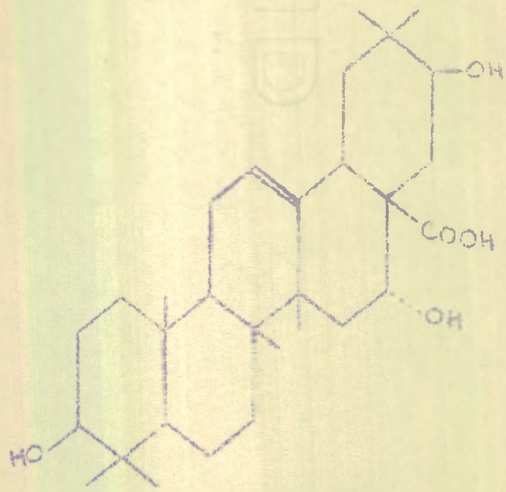


Fig.6

It will not be out of place to state that acacic acid, a new triterpenic acid, has earlier been obtained by Farooq, Varshney and Naim[195] from the bark of acacic irstia willd, in addition to lupeol and acacicl[197] and has been reported to be a trihydroxy monocarboxylic acid, belonging either to the group of α-amyrin or tetracyclic triterpenes[196]. But on the biogenetic grounds, Varshney[198] has proposed that this compound may have the following structure (CLXXXIV).



(CLXXXIV)

4. <u>The study of the seeds of Albizzia odoratissima Benth from Uttar Pradesh.</u>

For further work on the saponin and sapogenins from Albizzia odoratissima,Benth. an attempt was made to obtain these seeds from various sources, as very limited quantity of these seeds was available from the Silvicultu-

rist Maharashtra,Poona. After considerable difficulty a supply of these seeds of Albizzia odoratissima (Kala siris) was obtained from the Silviculturist U.P., Nainital. A study of these seeds from Uttar Pradesh showed that they differed considerably in shape and other characters from the seeds of Maharashtrian origin, already studied (Page 110) and therefore the investigation of these seeds was also undertaken.

The seeds of Albizzia odoratissima Benth obtained from the Silviculturist U.P. Nainital, were well defatted with light petroleum ether and the defatted seed powder was extracted with ethanol. The crude saponin was obtained in a manner described earlier (Cf. study of Albizzia odoratissima Benth seeds of Maharashtrian origin p. 114)

Finally the saponin was purified by extraction with n-butyl alcohol following the method of Wall et al [41] The n-butyl alcohol on recovery left a semi-solid mass. It was dissolved in alcohol and decolourised with activated charcoal and precipitated thrice with acetone. This gave a colourless non-hygroscopic powder giving all the tests for saponin. Though the saponin did not crystallise, it came down from hot n-butyl alcohol solution on cooling as colourless non-hygroscopic powder melting at 180-82°.

The saponin m.p.180-82° was chromatographed on Whatman filter paper No.1 using ethyl acetate:acetic acid:

water (3:1:1.5) as solvent mixture. The chromatogram on development with stannic chloride reagent[49] showed the presence of two spots, one major and the other minor. The saponin was also tested electrophoretically utilising Arches No.302 filter paper at 310 volts. Horizontal technique was applied using sodium tetraborate as buffer solution.[199] It also showed the presence of two saponins.

As the product obtained was a mixture of two saponins it was purified by acetylation and deacetylation. The saponin m.p.180-82° was acetylated with acetic anhydride in pyridine solution. The contents were left overnight, and then added dropwise to a large volume of ice cold water. It gave a colourless water insoluble saponin acetate, which was purified by dissolving in chloroform and precipitating by addition to petroleum ether. The operation was repeated three times which gave a colourless acetate of the saponin melting at 154-56°.

The saponin acetate m.p.154-56° was deacetylated by treatment with aqueous sodium hydroxide solution for twenty four hours at room temperature. The saponin acetate which was initially insoluble in water slowly became quite soluble in about two to two and a half hours contact with the alkali. After the contact of the desired time, the saponin was deionised by passing through a column of Amberlite IR 120(H) ion-exchange resin. The aqueous

saponin solution obtained was mixed with large amounts of dry n-butyl alcohol and finally distilled under reduced pressure to remove water. It left a colourless powder which was further purified by precipitation of an alcoholic solution by addition to a large volume of acetone and by crystallisation from hot n-butyl alcohol. On repeated crystallisations from hot n-butyl alcohol it gave a colourless product which actually came down on cooling as a powdery substance m.p.227-28°. It gave all the tests for saponins.[22]

The purity of the saponin m.p.227-28° thus obtained was established by paper chromatography of the saponin using Whatman filter paper No.1 and ethyl acetate: acetic acid:water (3:1:1.5) as solvent mixture. Ascending technique was utilised and the running time for the chromatogram was five hours. The spray reagent utilised was stannic chloride solution.[49] The paper chromatography showed that the saponin is a single entity.

The purity of the saponin was further established by the horizontal paper electrophoresis of the saponin on Arches filter paper No.302 and using sodium tetraborate 8.54 gm/lit as buffer[199] at 310 volts. The paper strip was sprayed by stannic chloride reagent and heated to develop the spots. The electrophoresis also showed that the saponin is a pure product as only one spot was visible this time.

Study of sapogenin: Aqueous solution of saponin was hydrolysed with sulphuric acid by heating on a water bath followed by refluxing on a heating mantle. A precipitate of the sapogenin obtained was filtered, washed a number of times with water free of acid and dried.

The genin obtained was refluxed with an alcoholic solution of potassium hydroxide. The alkaline solution was diluted with large amount of water and extracted with ether a number of times to remove any neutral sapogenin present. The ether on recovery did not give any product. The acidification of alkaline solution with hydrochloric acid after removal of any dissolved ether on a water bath, precipitated a colourless sapogenin which was filtered, washed with water free of hydrochloric acid. The acid genin could not be crystallised and therefore was acetylated with pyridine and acetic anhydride in cold for 18 hours. On crystallisation from methanol the acetate was obtained as colourless needles melting at 265-76°. (I.R. Spectra. Fig.7.)

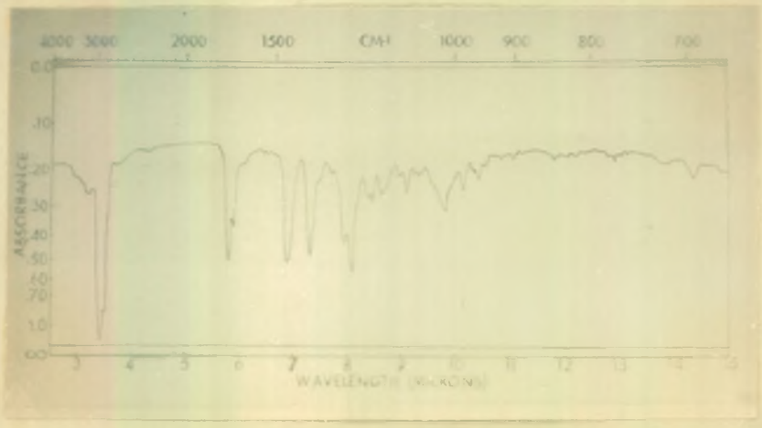


Fig.7

The acetate m.p.265-76° on deacetylation with methyl alcoholic potassium hydroxide, easily gave the genin back, which was crystallised from isopropyl alcohol as colourless shining crystals melting at 296-99° (I.R. spectra, Fig.8). It gave the positive Liebermann-Burchard reaction and all the colour tests of triterpenes. Both the genin and the acetate gave positive tetranitromethane test for unsaturation confirming the presence of atleast one carbon-carbon double bond in thegenin and acetate. The microanalyses of the genin and acetate showed the presence of two hydroxyl groups and one carboxyl group in the pentacyclic triterpenes

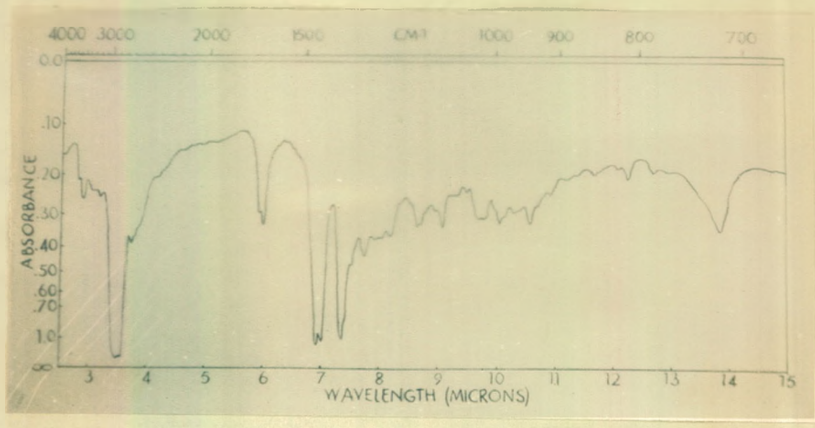


Fig.8

The presence of the carboxyl group was also readily detected by the formation of methyl ester m.p.218-15° (I.R. Spectra, Fig.9), on treatment of the genin with diazomethane The hindered character of the carboxyl group was evident by

the inertness of the genin to Fischer-Speier conditions of esterification. It also suggested the fact that the carboxyl group in this compound may be tertiary i.e. attached to C-17. The acetate on methylation with diazomethan gave a diacetyl methyl ester m.p.201-202° (I.R.Spectra, Fig.11). The elementary analysis of the ester of the genin and the acetyl methyl ester showed the genin to be a dihydroxy mono-carboxylic triterpenic acid.

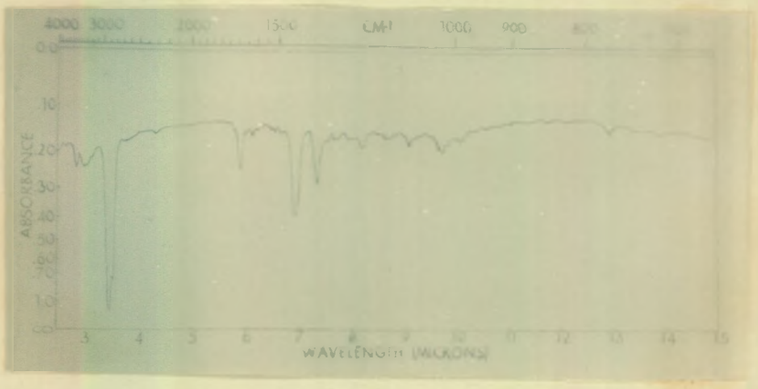


Fig.9

The position of the double bond with respect to the carboxyl group was deduced by the ready formation of a highly crystalline bromo-lactone of the acetate, m.p.180-81° (I.R. Spectra, Fig.10) through bromine in acetic acid from the diacetate. This showed

the compound to be either $\beta\gamma$ or $\gamma\delta$ unsaturated acid. The easy formation of the bromolactone in very good yield is indicative of olene-12-ene structure (Cf.King et al[107,184] Jeger[87]). The infra-red spectra of genin (Fig.8), acetate (Fig.7), methyl ester (Fig.9), acetyl methyl ester (Fig.11) and bromolactone (Fig.10) are all in conformity with these findings.

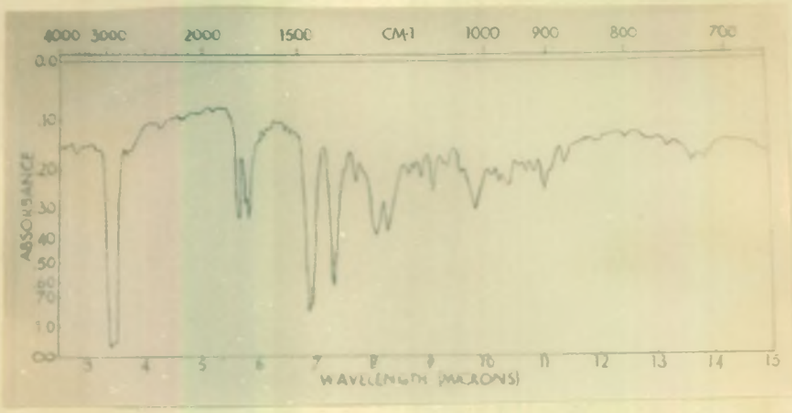
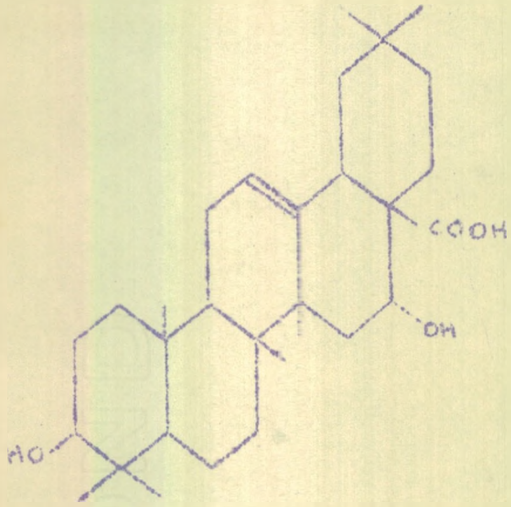


Fig.10

The comparison of the physical constants of the genin and its derivatives with all the known acids of β -amyrin group (table No.II) indicated the present genin to be identical with echinocystic acid (CLXXXV). The mixed melting point of the genin and the derivatives with the corresponding authentic samples of echinocystic acid and derivatives (table XII) and infra-red spectra (Fig.11) confirmed the present genin's identity as echinocystic acid (3β , 16α -dihydroxy Δ^{12} -oleanene-28-oic acid

or 16 α -hydroxy-oleanolic acid).



(CLXXXV)

TABLE XII

	213 Echinocystic fabacea	188 212, Albizzia lebbek	Albizzia Odoratissima present study
Echinocystic acid	305-12°	291-93°	296-99°
Diacetate	272-75°	248-50°	265-76°
Methyl ester	212-15°	210-11°	213-15°
Diacetyl methyl ester	198-201°	198-200°	201-202°

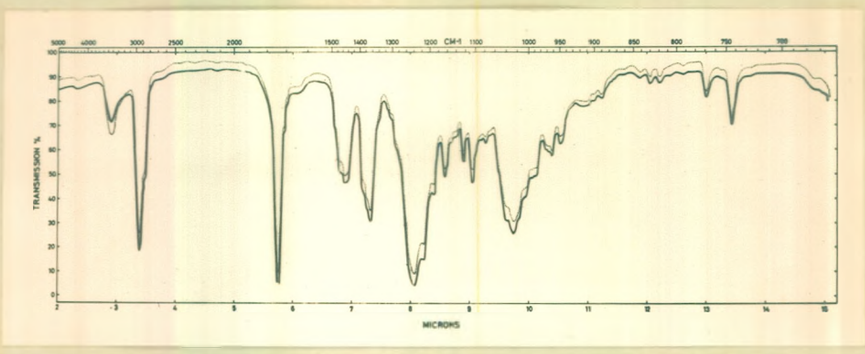


Fig. 11

. . . . . I.R.Spectra of Acetyl Methyl ester.

——————— I.R.Spectra of Acetyl Methyl Echinocystate.

Study of the saponin: After fixing the structure of the aglycone part of the saponin as echinocystic acid (3β, 16 α -dihydroxy oleanene Δ^{12} 28-oic acid (CLXXXV), it became necessary to find out the sugar moieties as well.

The sulphuric acid hydrolysate of the saponin was neutralised with freshly precipitated barium carbonate. The precipitate of barium sulphate was filtered and washed a number of times with hot water. The filtrate and the washings containing the sugars were evaporated to dryness in a vacuum oven at 35-40°. Another portion of the hydrolysate was neutralised by passing the sugar solution

through a column of Amberlite IRA 400 ion-exchange resin and evaporated to dryness in a vacuum oven. The concentrated sugar syrups obtained by both the methods were dissolved in a few drops of water and chromatographed on Whatman filter paper No.1 alongside with authentic sugars, using butanol:ethanol:water (40:11:19) as solvent mixture and utilising the descending technique. The spray reagents used were p-anisidine phosphate[214] and aniline hydrogen phthalate.[215] To confirm the absence of Keto-hexoses, urea-HCl spray reagent[216] was utilised. This showed the presence of the following four sugars:Glucose, arabinose, xylose and rhamnose. (Fig.12).

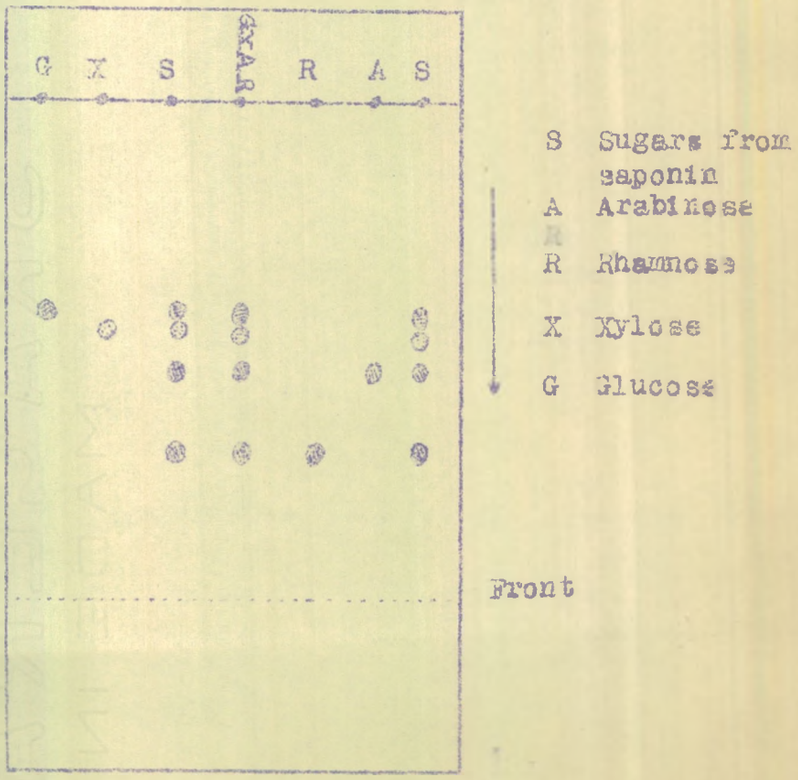
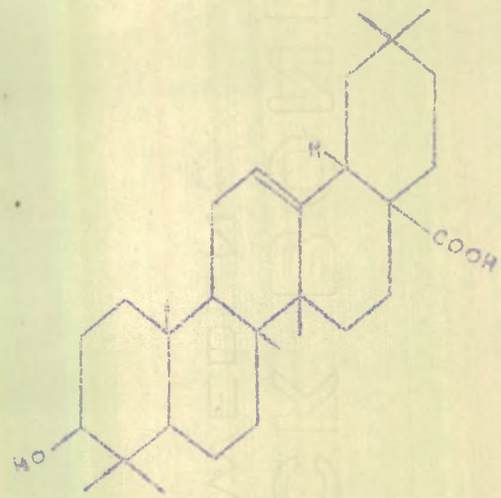


Fig. 12

The attempts at the partial hydrolysis of the saponin were unsuccessful. It may be concluded that the present saponin is a tetraglycoside of echinocystic acid containing glucose, arabinose, xylose and rhamnose as sugar moities. As it is a new saponin which has not been reported earlier in the literature, it has been named as Odoratissimin.

5. Study of the seeds of Albizzia procera, Benth. from Maharashtra.

The Albizzia procera Benth-seeds obtained from Madhya Pradesh as reported earlier, were recently studied by Farooq, Varshney and Hasan[184] in these laboratories for their saponin and sapogenin contents. They have been found to contain a saponin, Proceranin, which on hydrolysis yields a sapogenin, identified as Machaerinic acid (3 β - 21β - dihydroxy Δ^{12} 18β-oleanene-28-oic-acid) (CLXXXVI). In order to carry out further work on Proceranin, a supply of the seeds of Albizzia procera, Benth was obtained from the Silviculturist Maharashtra, Poona. On working with these seeds of Maharashtrian origin, the presence of a quite different genin than the one obtained earlier from Madhya Pradesh variety, was noted and therefore a study of the saponin and sapogenin from the Maharashtrian seeds was undertaken.



(CLXXXVI)

The usual treatment of the well defatted seed powder gave a saponin which was purified in the usual manner (Cf. page 114). It gave a colourless powder giving all the tests for saponin. The sulphuric acid hydrolysis of the saponin, easily gave a colourless sapogenin, which was transformed into its potassium salt. The ether extraction of the aqueous solution of the potassium salt gave no neutral genin. The ether extracted potassium salt solution on acidification with hydrochloric acid precipitated the acid genin which was transformed into acetate with pyridine and acetic anhydride in the cold. The acetate obtained was crystallised from methyl alcohol in colourless needles melting at 288-90°. Its analysis showed it to have the formula

$C_{32}H_{48}O_4$. The tetranitromethane test for the double bond was positive.

The infrared spectra (Fig.13) of the acetate showed an intense band at 8.05 μ indicative of the presence of an acetyl group. The non-existance of any sharp absorption in the 3 μ region clearly showed the absence of free hydroxyl group in the acetate. The presence of a band at 5.68 μ was clearly indicative of a lactone group. The infrared spectra of the acetate had a remarkable resemblance with the spectra of the pentacyclic triterpenic acids and not with those of the acetates of the steroidal sapogenins. It showed the absence of any sharp absorption in the region characteristic of the vinylidene group of the lupeol side chain and thus eliminated its relationship to lupeol group. The spectra showed more resemblance with the spectra of the members of the β -amyrin group than with those of the α -amyrin. It was therefore presumed that the acid belongs to β-amyrin-oleanolic acid group of pentacyclic triterpenes.

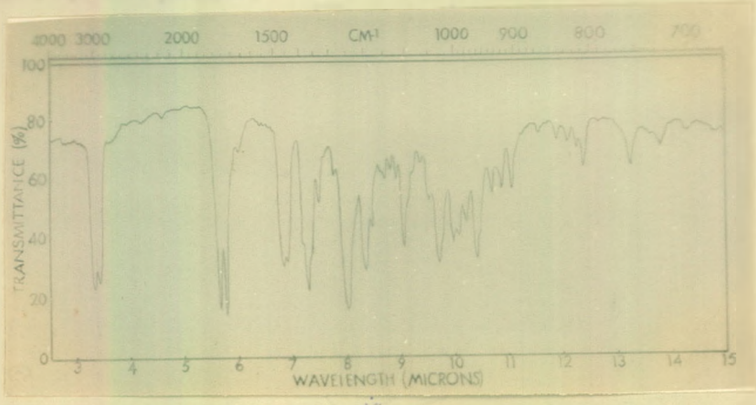


Fig 13

The presence of a lactone group as evidenced by the infra-red spectra was presumed to be between the acid and the second hydroxyl group and not between the carboxyl group and the double bond, as the tetranitromethane gave a positive test for the presence of the double bond in the acetate

The presence of lactone ring in which the acid group has taken part was further confirmed by the non-formation of acetyl methyl ester on treatment of the acetate m.p.288-90° with diazomethane. The acetate was recovered unreacted and was confirmed by mixed melting point and infra-red spectra with the acetate i.e. the starting material.

The acetate on deacetylation in the usual manner with methyl alcoholic potassium hydroxide yielded an acid genin crystallised from methyl alcohol as colourless needles melting at 268-72°. The infra-red spectra of the acid genin (Fig.14) showed the presence of free hydroxyl group. The presence of lactone group was not evident here as the lactone band moved to the region of carboxyl group at 5.9μ. This led to the conclusion that the acid group is forming a lactone with one of the hydroxyl groups in the acetate and is broken when the hydrolysis of the acetyl group is attempted. It gave a positive tetranitromethane test indicative of a carbon carbon double bond. The

Liebermann-Burchard reaction and other colour reactions for triterpenes were positive. The analysis of the acetate is also in agreement with this formulation of a lactone between the carboxyl and a hydroxyl group.

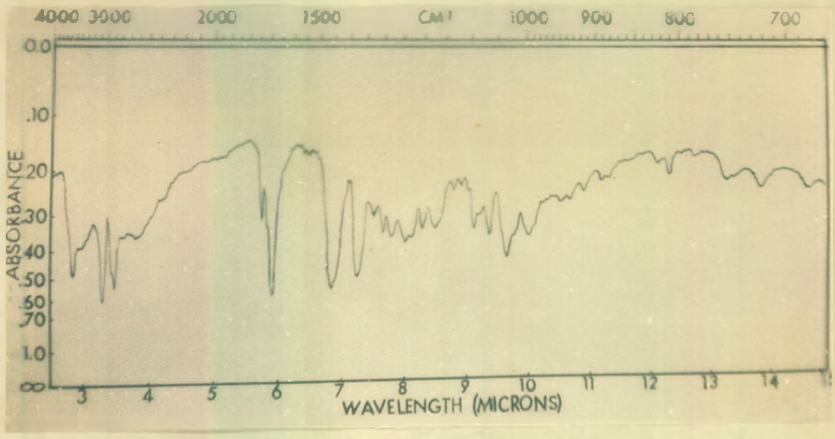


Fig. 14

The carboxyl group was fixed up by the formation of a methyl ester on treatment of the free acid genin with diazomethane in ethereal solution. The methyl ester crystallised from methyl alcohol as colourless needles melting at 224-27°. It also showed the presence of a carbon-carbon double bond with tetranitromethane. The infra-red spectra (Fig.15) also confirmed these findings.

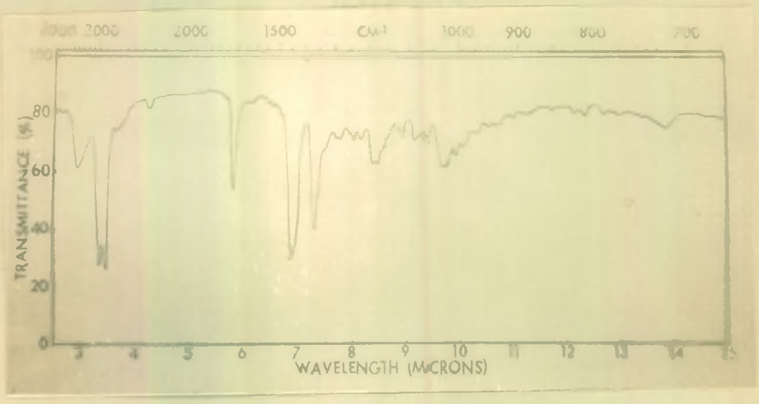


Fig. 15
I.R. Spectra of Methyl ester

The acetylation of the methyl ester with pyridine and acetic anhydride in cold, easily gave acetyl methyl ester which crystallised from methyl alcohol as colourless needles melting at 281-82°. (I.R. Spectra Fig.16). The micro-analytical results of the acetyl methyl ester and the methyl ester showed the genin to be a dihydroxy monocarboxylic acid with the formula $C_{30}H_{48}O_4$.

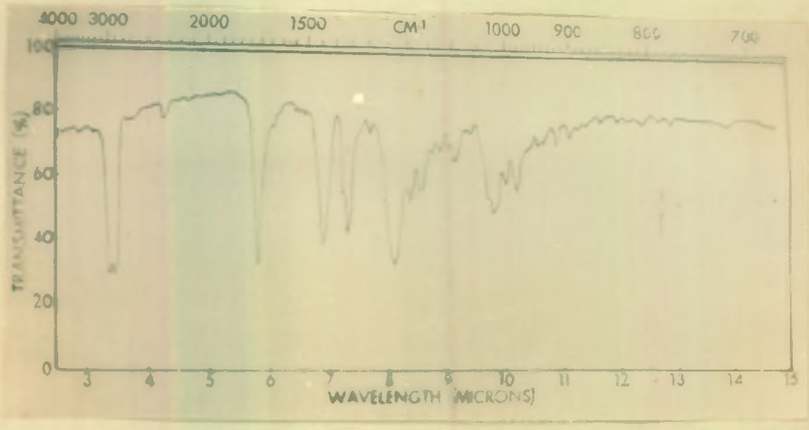
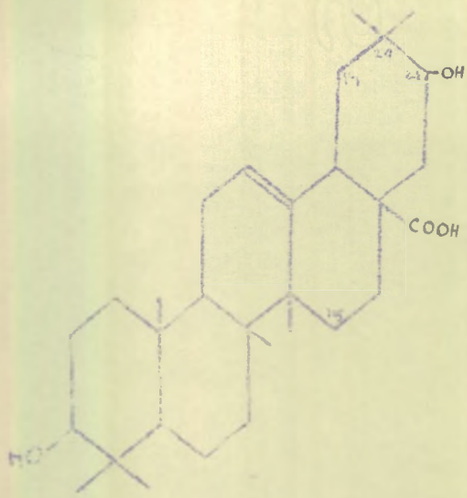


Fig. 16

All attempts to form acetyl bromo-lactone starting with the acetate were unsuccessful, which again confirmed that the acid group is not free in the acetate to take part in the formation of a bromo-lactone with the double bond or the acid does not belong to β-amyrin group. The second argument is more improbable in view of the Infra-red spectrographic evidence.

All these results are indicative that the present genin is a pentacyclic triterpenic acid with a formula $C_{30}H_{48}O_4$ having one carboxyl and two hydroxyl groups. By analogy with all the triterpenic acids of β-amyrin group one of the hydroxyl group has been assumed to be present at C-3 in ring A. The other hydroxyl group lactonises with carboxyl group in the process of acetylation of the genin yielding a 5 membered lactone ring (I.R.Spectra Fig.13) which places the hydroxyl group in γ-position to COOH group and therefore the positions 21,19 and 15 are only to be considered for the OH group. The physical constants of the present genin and the derivatives (Table XIII) are slightly higher than those of the corresponding derivatives of machaerinic acid (CLXXXVII) except the acetate which is quite different. As the other seeds from Madhya Pradesh have machaerinic acid, it was desirable to have the mixed melting points of all the corresponding derivatives (Cf. Table XIII).



(CLXXXVII)

TABLE XIII

	Machaerinic acid, m.p.	Present acid from Albizzia procera (Maharashtra) m.p.	Mixed m.p. of the two
Genin	256-58	268-72	268-70
Acetate	258-60	288-90	260-62
Methyl ester	224-25	224-27	225-27
Acetyl methyl ester	278-80	281-82	282-83
Diacetyl bromolactone	276-78	does not form.	

The methyl ester, acetyl methyl ester and the genin showed no depression in melting point when mixed melting point was taken, but the acetate, now fixed here as an acetyl lactone, showed a considerable depression in the melting point. This all confirmed our hypothesis that the lactonisation between one hydroxyl and a carboxyl group

takes place during the acetylation process of the genin.

The genin was then acetylated with pyridine and acetic anhydride with varying times at room temperature. Even in the acetylation which was attempted with one hour reaction time, the resulting product was a monoacetyl lactone. Acetylation with acetic anhydride and sodium acetate as well as acetic anhydride and pyridine in hot, also resulted in the formation of an acetyl lactone.

It was concluded therefore that the present genin is a pentacyclic triterpenic acid with two hydroxyl and one carboxyl groups at C-3, C-21, and C-28 respectively and a formulation identical with that of machaerinic acid (CLXXXVII) with some conformational changes presumably in the rings D and/or E which are responsible for the easy formation of a lactone. These changes are not clearly detectable in the infra-red spectra and have no effect on the mixed melting points even. It has been noted in this series that some times there is no depression in melting points even when two different substances are mixed. As this acid now seems to be a new one, it has been tentatively named as Procergic acid and the saponin as Mahaprocin.

As all these means failed to give any definite results, a resourse was taken to n-m-r- spectrography. The n.m.r. studies are also inconclusive in this respect

(Fig.17). The vinylic hydrogen absorption here is rather strange falling at 62-65 c.ps. region. The fact that the compound shows low absorption may mean that the double bond is conjugated. For example if there is a ketone at C-11 in addition to the double bond at C-12 such an absorption is obtained. It may be possible that the double bond is not at C-12 according to these findings of n.m.r. spectrography.

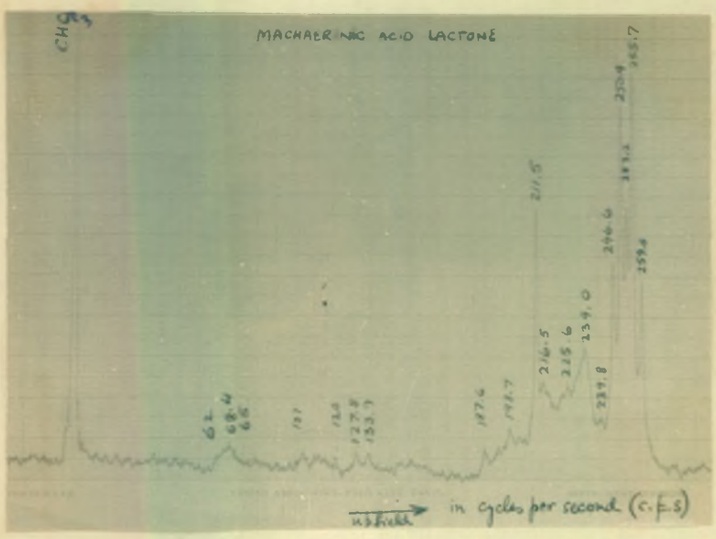
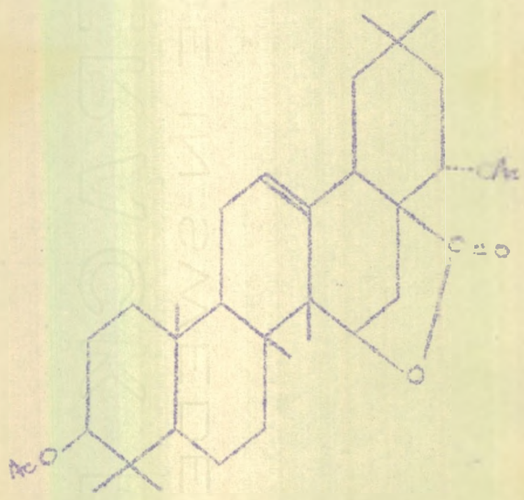


Fig. 17

The low absorption from 105-120 c.p.s. represents the alpha hydrogen of the C-3 acetate function. This absorption is about, where it should be.

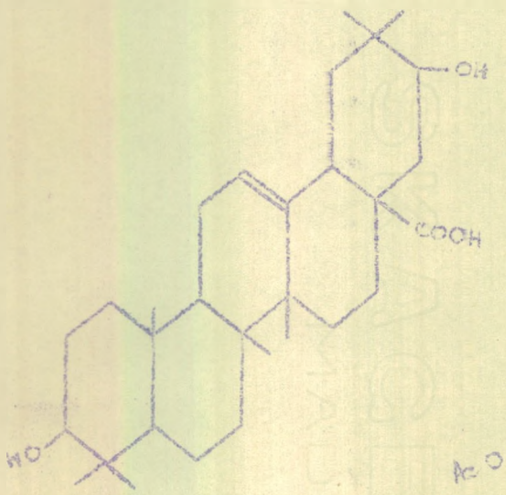
The doublet at 127.8 and 138.9 c.p.s. probably represents the alpha hydrogen of the alcoholic oxygen of lactone presumably taken to be at C-21. But again this absorption is much too high (i.e. upfield). For example, in dumortierigenin diacetate (CLXXXVIII), where a five membered lactone is present, the alpha hydrogen comes in at 104-120 c.p.s.



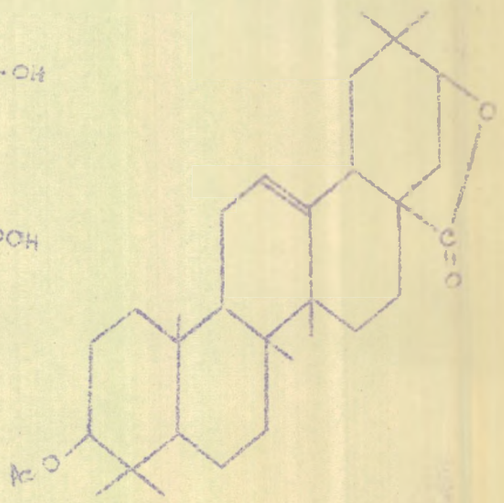
(CLXXXVIII)

The peak at 211.5 c.p.s. represents the methyl group of acetate function at C-3. The peaks at 246.6, 250.4, 253.2, 255.7 and 259.6 c.p.s. all represent saturated methyl functions. In other words there is no vinylic methyl group of the type $C=C-CH_3$. The peaks at the right hand side of the spectrum 187.6, 189.7, 225.6 c.p.s. etc. all stand for methylene or methine hydrogens.

In conclusion the following structures are tentatively proposed for procaric acid (CLXXXIX) and acetate (CXC) and that the OH at 21 and CCOH at 28 are in the same plane making the lactonisation possible.



(CLXXXX)

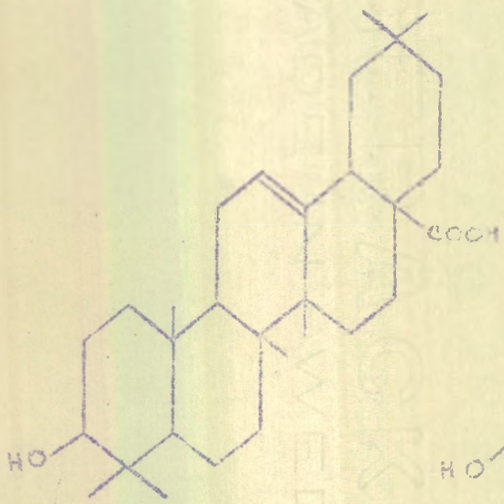


(CXC)

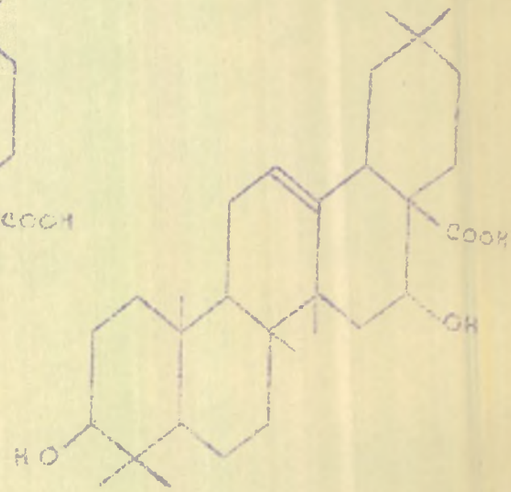
6. The study of the flowers of Albizzia lebbek, Benth from Uttar Pradesh.

As reported earlier, the seeds of Albizzia lebbek Benth from Utter Pradesh have been studied by Varshney et al[183,212] and found to yield a saponin from which oleanolic acid (CXCI) and echinocystic acid (CXCII) have been obtained. A number of other Albizzia species have

also been studied for saponin and sapogenin contents (Cf. page 110,124,135).



(CXCI)



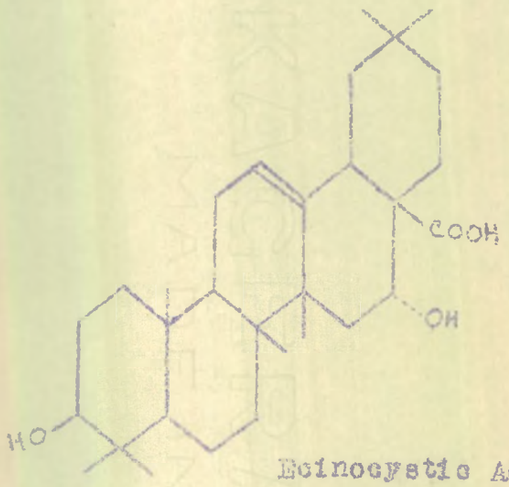
(CXCII)

A review to the literature showed that although much work has been done on the seeds from various species, no work seems to have been done on the flowers of any of the Albizzia species and therefore, the work on the flowers of Albizzia lebbek,Benth was taken up

The flowers of Albizzia lebbek Benth are, globose umbellate heads 1½ inches in diameter (excluding stamens), and are light yellow in colour with a fragrant odour,when fresh and odourless on drying. The flowers are used as a cooling medicine and also applied externally in boils, eruptions and swellings.[188,200]

The flowers of Albizzia lebbek Benth collected locally from the University campus yielded by alcoholic extraction and subsequent treatment (as given on page 114) a colourless hygroscopic powder giving all the tests for saponins. The saponin thus obtained was dissolved in large amount of water and hydrolysed with sulphuric acid by heating on a boiling water bath for one hour and thereafter by boiling on a heating mantle for another hour. A precipitate of the sarogenin was obtained, which was filtered, washed free of acid and dried. It was transformed into potassium salt. After removal of any neutral product by ether extraction the acid genin was precipitated by addition of hydrochloric acid. The genin was crystallised from methyl alcohol as colourless needles m.p.297-301°. The acetate obtained from the genin had m.p.257-62°. The acetate on esterification with diazomethane gave diacetyl methyl ester m.p.201-202°. It is a pentacyclic triterpenic acid belonging to β-amyrin group and has been identified as echinocystic acid (CXCIII) by mixed melting point with authentic samples of echinocystic acid and derivatives (Cf.echinocystic acid m.p.305-312°, acetate m.p.272-75°, acetyl methyl ester m.p.200-201°)[213]. The infra-red spectra of the acetyl methyl ester which

is superimposable with that of an authentic sample (Fig.18) confirmed its identity as echinocystic acid (CXCIII).



Echinocystic Acid

(CXCIII)

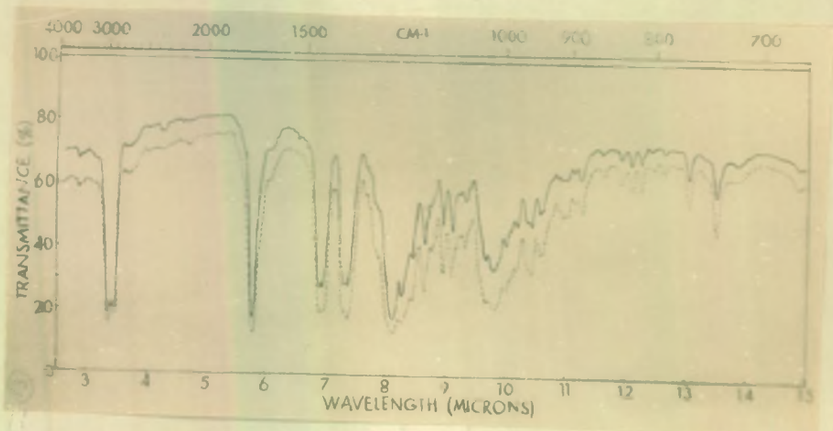
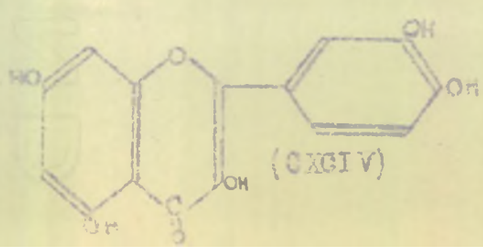


Fig. 18

Study of the anthoxanthin glycoside. The residue obtained after the evaporation of the alcoholic extract was dissolved in water and the solution extracted with n-butyl alcohol. The recovery of the butyl alcohol gave a mixture of the saponin and the glycoside. It was dissolved in alcohol, filtered and then added drop-wise to a large volume of acetone inorder to precipitate the saponin. The saponin was filtered off and the solution evaporated to dryness. This operation was repeated twice to remove the saponin. The acetone after the removal of the saponin was evaporated to dryness and the product obtained was tested for the presence of anthoxanthin glycosides. It gave salmon pink colouration with magnesium and hydrochloric acid.[201] The product could not crystallise and was therefore hydrolysed. The glycone obtained was then chromatographed using Whatman filter paper No.1 and acetic acid: water (60:40)[202] as solvent mixture. The spots were revealed by examination in ultra-violet light, ammonia vapour and ultra violet light, spraying with aqueous ferric chloride and sodium carbonate solutions.[202,203] It showed the presence of at least three different compounds. One of the components was identified as quercetin (CXCIV) by chromatography alongside with an authentic sample of quercetin.

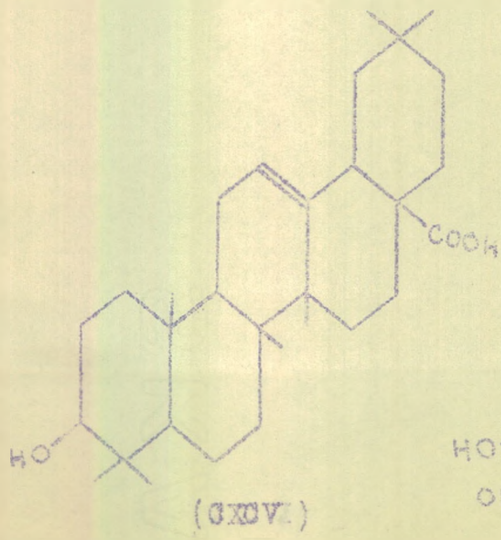


(CXCIV)

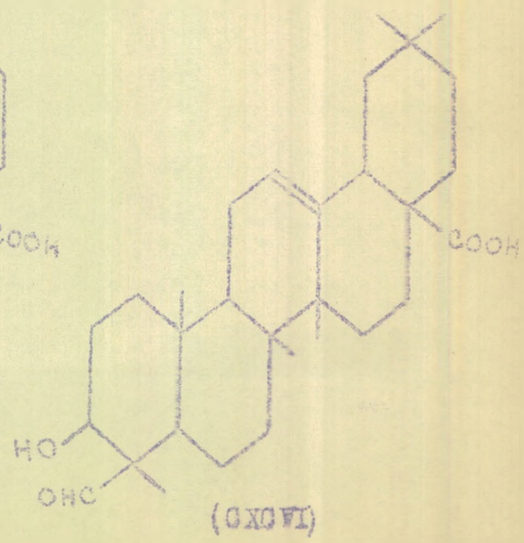
B. Cucurbitaceae

7. Luffa aegyptica Mill (Black variety).

Luffa aegyptica Mill locally known as "Ghia Torai" is a member of the family Cucurbitaceae. It is widely cultivated throughout north India and is commonly used as vegetable.[204] The other species of this plant, Luffa acutangula[205] and Luffa cylindrica[206] have earlier been studied and found to contain oleanolic acid (CXCV) while recently Djerassi and collaborators studied the seeds of Luffa operculata[207] and found that it contains a saponin giving on hydrolysis, gypsogenin (CXCVI) and an unidentified neutral genin (for which no physical constants have been mentioned in the literature).



(CXCV)



(CXCVI)

Although the presence of saponin in this variety, Luffa aegyptica, Mill., has been reported long ago [208] no work seems to have been done on the saponins and sapogenins from this plant. Rangaswami and Sambamurthy[209] have however isolated from the seeds of Luffa aegyptica, a bitter substance named amarin. Being engaged on the work on saponins and sapogenins it appeared of interest to take up the isolation and characterisation of saponins and sapogenins from Luffa aegyptica, Mill., seeds as well. Two varieties, black seeds and white seeds of Luffa aegyptica, Mill are available locally and the following part deals with the study of the saponins and the sapogenins of black seeds only.

The seeds of Luffa aegyptica Mill (Black variety) were procured from the local market as well as from M/S. H.Cooper & Co. Poona. The saponin was obtained from the concentrated ethanolic extract of the well defatted seed powder in the usual manner. It gave copious foam with water and all the tests for saponin. The saponin was dissolved in large amount of water and hydrolysed with sulphuric acid. It gave the sapogenin which was separated into an acid and a neutral fraction by sodium salt formation. After removal of the neutral genin by ether extraction, the acid genin was obtained by decomposing the aqueous solution of the sodium salt by hydrochloric acid. It was then transformed into acetate with sodium acetate

and acetic anhydride.

The acetate of the acid genin was separated into two completely different substances, by fractional crystallisation, melting at 266-68° and 176-81°. Both the substances gave the usual tests for triterpenes and positive Liebermann-Burchard reaction, and showed the presence of at least one carbon-carbon double bond with tetranitromethane.

The neutral genin, separated from the solution of the sodium salt of the acid genin by ether extraction was transformed into acetate and crystallised as colourless needles, m.p.262-64°. It gave all the tests for triterpenes but no yellow colour with tetranitromethane.

(a) Study of the acid genin acetate m.p.266-68°.

The deacetylation of acid genin acetate m.p.266-68° gave colourless fine needles of the acid genin m.p.302-304°. The genin was readily transformed into its methyl ester with diazomethane m.p.197-98°. The methylation of the acetate m.p.266-68° with diazomethane gave an acetyl methyl ester m.p.219-20°. All these derivatives showed unsaturation with tetranitromethane. The infra-red spectra of the acetate showed its relation to the β-amyrin group.

The comparison of the physical constants of the genin and its derivatives indicated the present genin to be id ntical with oleanolic acid (CXCV). Its identity with oleanolic acid was further confirmed by mixed melting point of the genin, acetate, methyl ester and acetyl methyl ester with authentic samples[183] and the comparison of the infrared spectra of the acetate with an authentic sample of oleanolic acid acetate, (Fig. 19), which are superimposable.

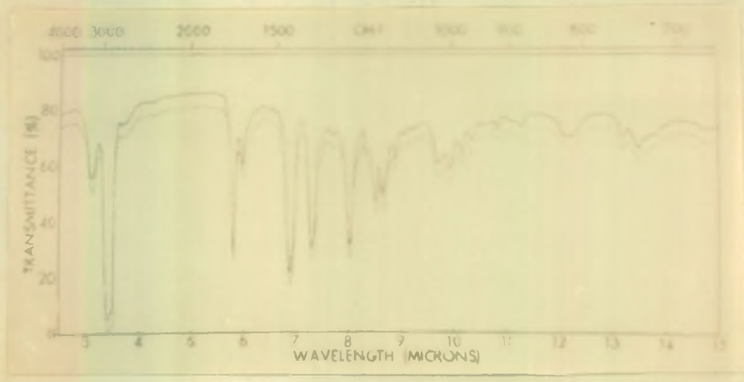


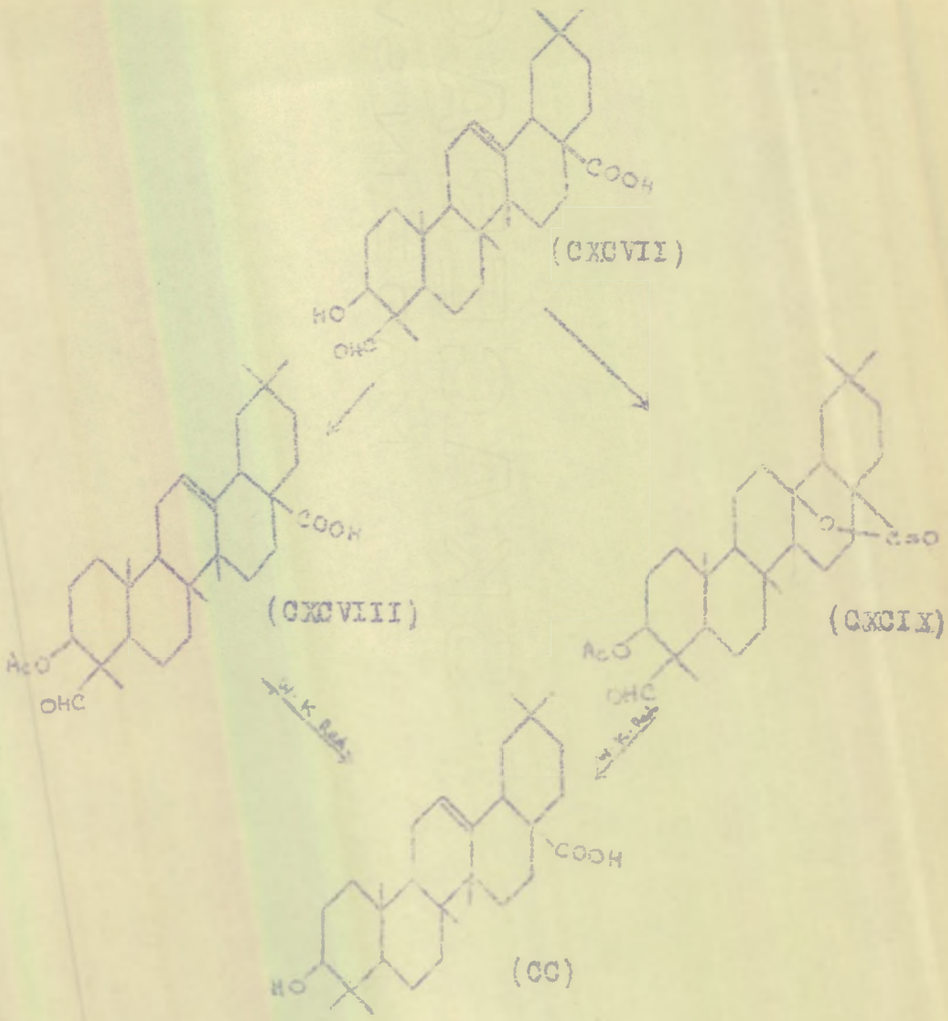
Fig. 19

(b) Acid genin acetate m.p.176-81.

The acetate was repeatedly crystallised, but no improvement in the melting point could be obtained. More crystallisation sometimes led to the isolation of the neut/ral.

genin acetate m.p.262-64° in very small quantities. The acetate m.p.176-81 gave positive Liebermann-Burchard and the tetranitromethane tests. The quantity of the second acid genin acetate was very small, but the neutral genin, which was isolated in the course of repeated crystallisation of this acetate threw some light on its constitution which is discussed under neutral genin.

Neutral genin: The neutral genin acetate m.p.262-64° which was obtained after its separation from the acid genins and cold acetylation, did not give any colour with tetranitromethane, showing the absence of a double bond. As a review to the literature of the other luffa species showed the presence of gypsogenin (CXCVI) in case of luffa operculata,[207] its presence was not out of place to predict in the present study also. Ruzicka and collaborators[210] have also encountered such a difficulty of getting two acetates in the case of gypsogenin m.p.176-77° and m.p.262° which they have shown, to be gypsogenin acetate (CXCVIII) and its lactone (CXCIX) respectively. The transformation of the acid genin into the neutral genin (lactone) suggested the presence of gypsogenin which was confirmed by comparison of the melting points from literature.



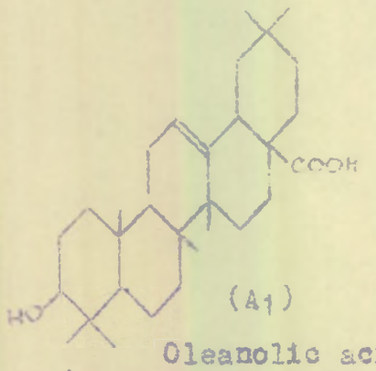
An attempt made to isolate cynsogenin, which is an aldehydic acid by the use of Girards P reagent was unsuccessful. Although Girard's P reagent is specific for ketones, it has also been utilized by Lederer and collaborators[211] to separate the aldehydes as well. Our attempts were unsuccessful and therefore the second acid genin acetate and the neutral genin were subjected to Wolff-Kishner reduction, giving genins m.p. 288-92 which wer

transformed into acetates m.p. 263.66° 264-66. The reduction product was identified as oleanolic acid. No other genin could be isolated after the reduction showing that the new acid genin and the neutral genin have been transformed into oleanolic acid(cc).

This showed that this plant contains gypsogenin and oleanolic acid, and that gypsogenin lactnises and gives lactone with the double bond, which is isolated as a neutral genin. Now the question arises, that when gypsogenin which has the same stereochemical skeleton as oleanolic acid can lactonise, why not the oleanolic acid lactonises with the double bond under similar conditions. Our all attempts to clarify this anamoly were unsuccessful and it is presumed that the stereochemical structure of gypsogenin should be different from oleanolic acid. But all the information available in the literature due to Ruzicka Jeger, Barton- Djerassi etc. shows that the skeleton of gypsogenin is same as of oleanolic acid and needs further substentiation.

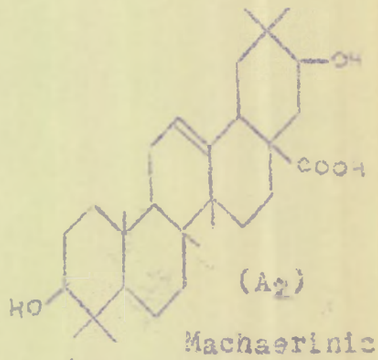
The following are the chemical structures of all the triterpenes which have been encountered in the members of the family Leguminosae, and Cucurbitaceae.

A- Family Leguminosae.



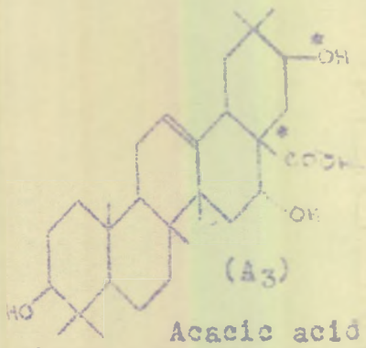
(A_1)

Oleanolic acid
(Sesbania aegyptica & S. aculeata).



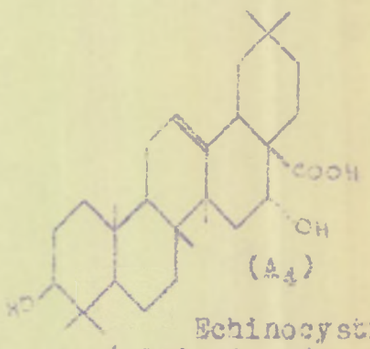
(A_2)

Machaerinic acid
(Albizzia odoratissima from Maharashtra).



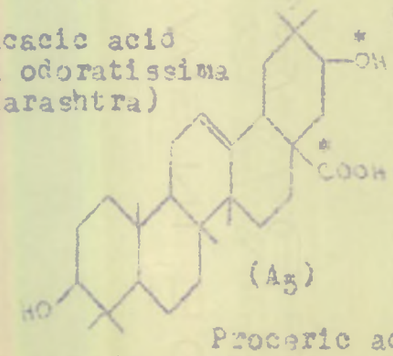
(A_3)

Acacic acid
(Albizzia odoratissima from Maharashtra)



(A_4)

Echinocystic acid
(Albizzia odoratissima from Uttar Pradesh)



(A_5)

Proceric acid
(Albizzia procera from Maharashtra)

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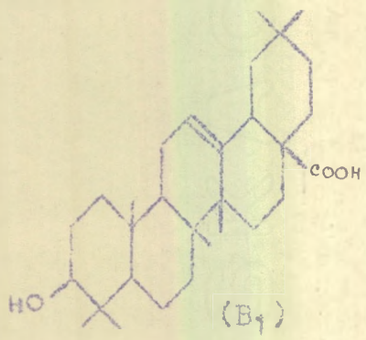
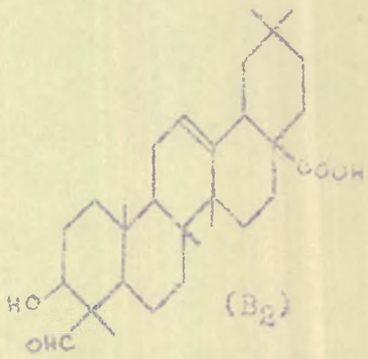
*Same plane making very easy lactonisation possible.

B - Family Cucurbitaceae

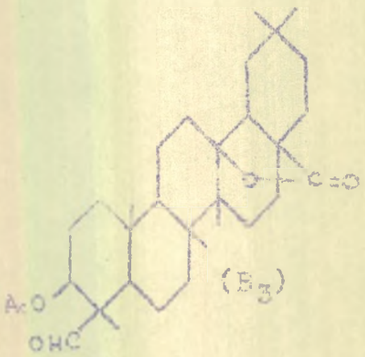
(Luffa aegyptica Mill. Black variety).



Oleanolic acid



Gypsogenin



Acetyl gypsogenin lactone.

The most interesting fact that emerges from the study of the family leguminosae is that all these compounds are the members of the β-amyrin series. Leaving aside the ubiquitous 3β-hydroxyl group, the hydroxylation has taken place in all these products isolated (from A_1 to A_5) in two positions i.e. 16 and 21 which fall in only two rings, D and E. A unique feature found in two of the members (A_3 and A_5) is that the orientation of the carboxyl group and one of the

hydroxyl group is the same making the lactonisation between them not only easy but extremely easy, which has not been encountered so far in any acids from any other family. Further there is another striking fact that all these genins occur as saponins and none has been noted in the free state, in this family. It appears that there exists a very close biogenetic relationship between these acids. But to ascertain the existance of any such relationship, it will be necessary to make a thorough study of other members of this family.

It is also to be noted that the locality from where the seeds are obtained, plays an important part on the nature of the constituents, chiefly on the oxygenation. The seeds obtained from two different localities are found to be different in the nature of sapogenins, differing in the position of oxygen attachment or in the orientation of the functional groups. Therefore, a new system of classification of the plants on the basis of their chemical constituents, also deserves consideration.

EXPERIMENTAL

E X P E R I M E N T A L

All the melting points reported in this thesis have been taken on Kofler's Hot Microscopical Stage and are corrected. The micro-analyses have been done at the Department of Chemical Technology, University of Bombay, Department of Chemistry, M.S. University of Baroda, or Drs. Weiler and Strauss, Oxford England.

The Infra-red spectra have been taken in Nujol at the National Chemical Laboratory Poona, (Grubb-Parsons Infra-red spectrometer), in this laboratory (Dr. I.P.Varsyney-Infra-red, Perkin-Elmer Model 137) and Laboratoire d'Infra-rouge, Universite de Paris, France (Prof. P.Barchewitz and Dr. L. Henry-Perkin-Elmer Model 21 in KBr plate). The Ultra-violet spectra have been taken in this laboratory in ethanol using Beckman ultraviolet spectrometer model DU. The nmr spectra have been taken at the Pennsylvania State University, U.S.A. (Prof. M.Shamma).

The electrophoresis have been done on Jouan semi-automatic electrophoresis apparatus model No.1603 and the paper chromatography at the room temperature.

2. Study of the seeds of Sesbania aculeata Pers.

Defatting: Well powdered seeds (500 gms) of Sesbania aculeata Pers were extracted with light petroleum ether (40-60). The recovery of the solvent left a greenish oil (18.2 gms).

Isolation of the saponin: The defatted seeds (250 gm) were exhausted with ethanol. On recovery of ethanol, an oily semisolid residue was obtained. It was successively treated with petroleum ether, ether, carbontetrachloride and acetone. This left a brown semisolid mass which was dissolved in a small quantity of alcohol and precipitated by addition to a large volume of ether. The process was repeated several times. Finally the precipitation was made by addition to a large volume of dry acetone. A light cream coloured powdery substance (17 gm) was obtained, which was filtered on a Buckner funnel, washed with acetone and dried in a vacuum desiccator.

Hydrolysis of the saponin. The saponin (7 gm) was dissolved in about three litres of water containing sulphuric acid (10%). The contents were heated on a boiling water bath for one hour, and finally the hydrolysis was completed by refluxing for another one hour. A brown precipitate (1 gm) was obtained, which was filtered and washed with water, till the washings showed no acidity. It was dried.

Separation into acid and neutral fractions.

The crude genin (2 gm) was refluxed with methanolic sodiumhydroxide (6%; 100 cc)for half an hour. Half of the solvent was recovered and the contents were then diluted with 1 litre of water. It was extracted several times with ether. The ethereal extracts were all combined and washed with water four times, till free of alkali.

The ether on recovery gave a colourless neutral genin.

The alkaline solution left after ether extraction gave on acidification, the acid genin which was filtered, washed with water and dried.

Acetylation of the acid genin

The acid genin (500 mg) was dissolved in pyridine (12 cc) and acetic anhydride (8 cc) was added to it. It was left for 24 hours and then poured drop by drop into a large quantity of ice cold water. A precipitate was obtained, which was filtered and washed with water free of pyridine and acetic acid.

On crystallisation from methyl alcohol, it gave colourless fine needles m.p.262-64°.

No depression in melting point was noted, when mixed melted with oleanolic acid acetate.

Acetyl methyl ester. The acid genin acetate (200 mg) was dissolved in ether 100 cc and treated with an excess of ethereal solution of diazomethane. After 14 hours contact with diazomethane, the excess of diazomethane and ether was removed by evaporating on a hot water bath. A colourless product was obtained which was crystallised from methyl alcohol containing a little chloroform. Colourless shining plates m.p. 220-21° were obtained. It did not depress the melting point when mixed melted with acetyl-methyl oleanolate.

Neutral genin acetate:

The neutral genin (300 mg) was acetylated with pyridine (15 cc) and acetic anhydride (10 cc) in the cold for 24 hours. After the required time, it was poured drop by drop, into water containing crushed ice. A white ppt. was obtained, which on crystallisation from methanol gave colourless crystals m.p. 185-90°.

3. Study of the seeds of Albizzia odoratissima Benth from Maharashtra

Defatting:

Well powdered seeds (250 gms) of Albizzia odoratissima,Benth obtained from the Silviculturist Maharashtra,Poona were extracted in a soxhlet apparatus with light petroleum ether (40-60°). The recovery of the solvent left a greenish yellow oil (15 gms).

Extraction of the Saponin. The defatted seed powder was dried and extracted with ethanol in a soxhlet apparatus The solvent was recovered on a water pump under reduced pressure, when a brown syrupy liquid was obtained. This residual mass was treated with petroleum ether, ether, carbontetrachloride, chloroform and acetone. The residue left over was dissolved in ethyl alcohol and filtered. The solution was added drop by drop,to a large volume of ether, which precipitated the saponin as a light brown substance. The precipitate was collected, redissolved in ethanol and precipitated with ether. The operation was repeated several times. The precipitate obtained was again dissolved in ethanol and precipitated by addition to a large quantity of dry acetone. This gave a light cream coloured hygroscopic powder giving all the tests for saponins - produced abundant foam on vigorous shaking with

water, was toxic to fishes in low concentrations and had high haemolytic action on the red blood corpuscles in very low concentrations.

Hydrolysis of saponin:

The cream coloured powder of the saponin (5 gm) obtained by the ether/acetone precipitation was dissolved in a large quantity of water and hydrolysed with 10% sulphuric acid by heating on a boiling water bath for an hour and finally by refluxing on a wire-guaze for another hour. After the completion of the hydrolysis it was allowed to cool. The precipitate was filtered, washed with water till the washings were neutral and finally dried. The genin thus obtained was dissolved in alcohol and decolourised with activated charcoal.

Purification of the genin:

The crude genin (5 gm.) was refluxed for half an hour with alcoholic potassium hydroxide solution (500 cc; 5%), and then half of the solvent was recovered under reduced pressure. The solution was diluted with water, (2 litres) and left overnight, but no solid product separated out. It was extracted five or six times with ether. The ethereal extracts were combined and washed free of alkali. On recovery of the ether, no appreciable quantity of the neutral genin was obtained.

The alkaline solution left after ether extraction was warmed on a boiling water bath in order to remove any amount of ether present in the solution. It was cooled, acidified with hydrochloric acid and left over for two hours. The precipitate formed was filtered, washed with water to remove the hydrochloric acid and dried. This gave the acid genin.

(v) Acetylation:

The acid genin (0.5 gm) was acetylated with acetic anhydride (20 cc) in presence of pyridine (40 cc). The contents were left overnight, and then poured dropwise in cold water with constant stirring, when a precipitate was obtained. It was filtered, washed with large quantity of water free of acetic acid and pyridine and dried.

On repeated fractional crystallisation from methyl alcohol containing a small amount of chloroform, following a triangular scheme, it was separated into two different acetates, which had melting points 260-65° and 228-34° quite different from each other. Both the substances gave yellow colour with tetranitromethane.

(vi) Acetate m.p.260-65°.

There was no depression on mixed melting with diacetyl machaerinic acid (260-64°). (Cf. Machaerinic acid diacetate m.p.258-60°)[184].

Analysis:

Found C 73.62% H 9.66%

Calc. for $C_{32}H_{52}O_6$ C 73.35% H 9.41%

I.R. Spectra ν^{nujol}_{max} 1723, 1689, 1257 cm^{-1}

The deacetylation of the acetate:

The acetate m.p.260-65° (300 mg) was refluxed for two hours with methyl alcoholic potassium hydroxide (150 cc 5%), and allowed to cool. It was diluted with a large amount of water (800 cc) and left overnight at the room temperature. No solid potassium salt separated out. The solution was acidified with hydrochloric acid, whereupon it yielded a colourless precipitate which was filtered, and washed with water free of the acid and crystallised from methanol. The genin came down in colorless needles m.p.266-70°. It gave positive test with tetranitromethane and positive Liebermann-Burchard reaction.

It showed no depression in melting point when mixed melted with machaerinic acid (Cf.machaerinic acid m.p.256-58°).[184]

Analysis:

Found C 76.19% H 10.37%

Calcd.for $C_{30}H_{48}O_4$ C 76.22% H 10.23%

I.R. Spectra ν^{nujol}_{max} 3400, 3240, 1741, 1689 Cm^{-1}.

Methyl ester: The acid genin (200 mg) was dissolved in ether (200 cc) and mixed with an excess of an ethereal solution of diazomethane. The contents were left overnight at room temperature. The excess of diazomethane was then removed on a water-bath. It left a residue which was crystallised from methanol as colourless needles m.p.224-25°. It gave a positive test for unsaturation with tetranitromethane. Mixed melting point with methyl machaerinate was 223-25° (Cf. Machaerinic acid methyl ester m.p.224-25°).[184]

I.R.Spectra $\nu_{max.}^{nujol}$ 3400, 1724 Cm^{-1}

Acetyl methyl ester:

The acetate (100 mg) dissolved in ether was treated with an excess of ethereal solution of diazomethane. After 24 hours contact, the ether was evaporated, and the product crystallised from methanol as colourless fine needles m.p.279-80°. It gave a yellow colour with tetranitromethane. Mixed melting point with diacetyl methyl machaerinate was 279-80° (Cf. diacetyl methyl machaerinate m.p.278-80°).[184]

Anal. Found. C 72.96% H 9.39%

Calcd.for $C_{35}H_{54}O_6$ C 73.64% H 9.53%

I.R. Spectra λ_{max}^{nujol} 5.82; 8.1 μ

Acetyl bromo lactone:

The diacetate (150 mg.) and sodium acetate (300 mg.) were dissolved in acetic acid (25 cc; 90%) and a solution of bromine in acetic acid (6 cc; 4%) was added to it drop by drop. The flask was left for an hour at the room temperature and thereafter the contents poured in water (300 cc) containing sodium thiosulphate (500 mg.). The precipitate formed was filtered, washed with water and crystallised from methanol as colourless crystals m.p.288-90°. It did not give a yellow colour with tetranitromethane. Mixed melting point with diacetyl bromolactone of machaerinic acid was 273-77° (Cf. diacetyl bromolactone of machaerinic acid m.p.276-78°).[184]

Analy.	Found.	C 64.09%	H 8.25%
Calcd. for	$C_{34}H_{51}O_6Br$	C 64.23%	H 8.08%

Acetyl methyl ester of the acetate m.p.228-34°.

The acetate m.p.228-34°, was treated with an excess of ethereal solution of diazomethane. After 24 hours contact, the excess of ether and diazomethane was evaporated on a water bath. The residue obtained was crystallised from methyl alcohol in colourless needles m.p.232-34°. It did not depress the melting point when mixed melted with the original sample of the acetate. Mixed melting point with the acetate of acacic acid was 232-35° (Cf. acacic acid

acetate m.p.234-36°).[195]

I.R.Spectra λ_{max}^{nujol} 5.63; 8.02 μ

Reduction of the lactone acetate m.p.228-34° with lithium aluminium hydride:

The substance (600 mg) was dissolved in ether (200 cc) and to it was added lithium aluminium hydride (7 gm) in small portions at a time with stirring of the solution. The contents were left for 2 days at room temperature. Excess of $LiAlH_4$ was destroyed by addition of ethyl acetate in small quantities, and then finally, it was diluted with water and extracted with ether four or five times. The ethereal layers were combined together and washed with water, till free of alkali. On recovery of the ether, a product was obtained, which was crystallised from methanol as colourless crystals m.p.295-98°. It did not depress the melting point when mixed melted with the tetrol[196] obtained from acacic acid acetate. It gave a yellow colour with tetranitromethane.

4. Study of the seeds of Albizzia odoratissima Benth. from Uttar Pradesh.

Defatting: Well powdered seeds (400 gms) of Albizzia odoratissima Benth obtained from Nainital (U.P.) were exhausted in a soxhlet apparatus with light petroleum ether (b.p.40-60°). The recovery of the solvent left an oil greenish yellow in colour (18 gms).

Extraction: The defatted seed powder was exhausted with 95% alcohol in a soxhlet apparatus and the solvent recovered under reduced pressure, which left a brown syrupy liquid. It was dissolved in alcohol and filtered. The alcohol was evaporated to dryness, the residue was extracted with ether, petroleum, ether, chloroform, carbontetrachloride and acetone inorder to remove the impurities soluble in these solvents. The residue was dissolved in alcohol and added dropwise to a large volume of ether/acetone , when a light brown precipitate was obtained. The proces of dissolution and precipitation was repeated several times, which gave a cream coloured hygroscopic powder of the saponin.

Purification of the saponin:

The saponin obtained from the above procedure was dissolved in water containing sodium chloride (5%). The pH of the solution was adjusted between 4 and 5 by the addition of an appropriate quantity of hydrochloric acid.

It was extracted four times with water saturated n-butyl alcohol.[41] The butanol layers were combined together and washed with 5% sodium chloride solution. The butanolic solution of the saponin was recovered under reduced pressure. In the end some more butanol was added and recovered under reduced pressure inorder to remove any amount of water remaining in the flask. A semi solid mass was left behind, which was dissolved in alcohol and decolourised with activated charcoal. The alcoholic solution of the saponin was concentrated to a small volume and the saponin precipitated by addition to a large volume of acetone. On crystallisation from hot n-butyl alcohol, a colourless powdery substance m.p.180-82° was obtained on cooling of the solvent. It gave all the tests for saponin, such as abundant foam with water in very dilute solutions, sneezing, toxicity to fishes in low concentrations, strong haemolytic effect on red blood corpuscles in dilute concentrations and specific color reactions. This showed two spots, one major and one minor on paper chromatography and electrophoresis as described later.

(iii) Acetylation of the saponin:

To a solution of the saponin (700 mg) in pyridine (25 cc), acetic anhydride (15 cc) was added with shaking. The mixture was left overnight and then added drop by drop to a large volume of ice cold water, which gave a

precipitate of the saponin acetate. It was filtered, washed well with water and dried. A colourless product, thus obtained was dissolved in a few ccs of chloroform and added dropwise with stirring to light petroleum ether (400 cc). This gave a solid product which was filtered, redissolved in chloroform and reprecipitated by addition to petroleum ether. The process of dissolution in chloroform and precipitation with petroleum ether was repeated three times. It gave a colourless powder of the saponin acetate melting at 154-56°.

Analysis. Found: C 57.0% H 6.8%

Deacetylation of the saponin acetate:

The saponin acetate (700 mg) was shaken with sodium hydroxide solution (0.4 N; 150 cc) for one hour continuously and then left for one day with occasional shaking. Slowly the acetate decomposed to give the saponin, which went into the solution. The alkaline solution of the saponin was neutralized by passing through a column of Amberlite IR-120 (H) ion-exchange resin. The neutralised saponin solution was mixed with large quantity of butyl alcohol and distilled under reduced pressure. More butyl alcohol was added and distilled under vacuum till there remained no water in the flask. A solid residue was left over, which was dissolved in a small quantity of alcohol and precipitated with acetone. It was filtered. The saponin thus obtained

was dissolved in hot butyl alcohol, from which it separated as colourless powder on cooling. It melted at 227-28°, and gave all the tests for saponin.[22]

Analysis Found: C 51.6% H 8.1%

Paper chromatography of the saponin:

A small amount of saponin was deposited on a Whatman filter paper No.1 and chromatographed for five hours using ascending technique and ethyl acetate:acetic acid:water (3:1:1.5) as solvent mixture. The chromatogram was dried in the air, sprayed with stannic chloride reagent ($SnCl_4$:AcOH:CCl_4 - 3:25:25)[49] and heated in an oven at 100° for five minutes. It showed only one spot of the saponin.

Electrophoresis:

A small quantity of the saponin was dissolved in a few drops of alcohol and deposited on a strip of filter paper Arches No.302. Horizontal electrophoresis was done on a Jouan apparatus using borate buffer (sodium tetraborate 9.54 gms/litre)[199] at 310 volts for eight hours. After drying, the paper was sprayed with stannic chloride[4] reagent and heated in an oven at 100° for 5 minutes. It showed that the saponin is a pure product as only one spot was obtained. On recording a graph on the same apparatus, only one peak was obtained, which further proved that the saponin is a single entity.

Isolation of the Sapogenin:

The saponin (2 gm) m.p.227-28° obtained by the deacetylation of the saponin acetate was dissolved in water (2 litre) and hydrolysed with sulphuric acid (10%) by heating the solution first on a boiling water bath for one hour and then completing the hydrolysis by refluxing the solution for two hours more. After completion of the hydrolysis, the precipitate of the genin obtained was filtered and washed with water till the washings were neutral.

Separation of acid and neutral sapogenins.

The genin (2 gm) was converted into its potassium salt by refluxing with methyl alcoholic caustic potash solution (5%; 100 cc). Half of the solvent was then distilled off and the solution diluted with large amount of water. It was extracted three or four times with ether. The ethereal extracts were combined together and washed with water free of alkali. On recovery of the ether no neutral sapogenin could be obtained.

The alkaline water solution left after ether extraction was heated on a water bath in order to remove any amount of ether present. It was cooled and acidified with hydrochloric acid. The precipitate obtained was filtered, washed with water free of the acid and dried. It gave a colourless acid genin.

Acetylation of the acid genin:

The genin (300 mg) was dissolved in pyridine (15 cc) and acetic anhydride (10 cc) was added to it. It was left at room temperature for 18 hours. The reaction mixture was poured in ice cold water drop by drop. The acetate which precipitated as a colourless product, was left overnight and then filtered. It was washed a number of times with water to remove pyridine, dried and crystallised from methyl alcohol into colourless needles m.p. 265-76°. It gave a positive reaction with tetranitromethane. The mixed melting point with an authentic sample of diacetyl echinocystic acid was undepressed.

Analysis. Found: C 73.0% , H 9.4%

Calc.for $C_{34}H_{52}O_6$ C 73.3% H 9.4%

I.R. Spectra $\lambda^{nujol}_{max.}$ 5.81, 5.90, 8.09 μ

Deacetylation of the acetate:

The acetate (500 mg) was refluxed for two and a half hours with methyl alcoholic potassium hydroxide solution (potassium hydroxide 5 gms in 100 cc methyl alcohol). Half of the solvent was recovered and the solution diluted with water (400 cc). The contents were left when no solid potassium salt of the genin separated out. The solution was acidified with hydrochloric acid which precipitated the acid genin as colourless product. It was

filtered, washed with water free of hydrochloric acid and crystallised from isopropyl alcohol as colourless plates m.p.296-98°. It gave a positive test for double bond with tetranitromethane a positive Liebermann-Burchard reaction and all the colour tests of triterpenes. Mixed m.p. with echinocystic acid was 296-98°.

Analysis. Found: C 74.4% H 9.8%

Calc.for $C_{30}H_{48}O_4$ C 76.2% H 10.2%

Calc.for $C_{30}H_{48}O_4, C_3H_7OH$ C 74.7% H 10.5%

I.R. Spectra: $\lambda_{max.}^{nujol}$ 2.87; 5.97 μ

Methyl ester:

The acid genin (300 gm) was dissolved in ether (200 cc) and an excess of ethereal solution of diazomethane was added to it. The solution was left overnight. The unreacted diazomethane, along with ether was removed on a water bath. The residue left over in the flask was crystallised from methyl alcohol as colourless needles melting at 213-15°. It gave a yellow colour with tetranitromethane. Mixed melting point with authentic sample of methyl echinocystate was undepressed.

Analysis. Found: C 74.9% H 9.8%

Calc.for $C_{31}H_{50}O_4$ C 76.49% H 10.36%

Calc.for $C_{31}H_{50}O_4.CH_3OH$ C 74.07% H 10.4%

I.R. Spectra: $\lambda_{max.}^{mujol}$ 2.85, 5.90 μ

Acetyl methyl ester:

The acetate (200 mg) was dissolved in ether (300cc) and an excess of diazomethane in ethereal solution was added to it. After a contact of twenty four hours the ether and the excess of diazomethane was evaporated on a water bath. The product obtained was crystallised from methyl alcohol as colourless needles melting at 201-202°. It showed unsaturation with tetranitromethane. Mixed melting point with authentic sample of diacetymethyl echinocystate was undepressed.

Analysis. Found: C 73.5% H 8.9%

Calc.for $C_{35}H_{54}O_6$ C 73.6% H 9.5%

I.R. Spectra: $\lambda_{max.}^{mujol}$ 5.75, 8.05 μ

Acetyl bromo lactone:

The acetate (100 mg) and sodium acetate (200 mg) were dissolved in acetic acid (20 cc; 90%) and a solution of bromine in acetic acid (4%; 5 cc) was added to it dropwise. It was confirmed that after the reaction, still an

excess of bromine was present. The contents were left for one hour and then poured drop by drop in water (300 cc) containing sodium thiosulphate (500 mg). The precipitate obtained was filtered, washed with water and crystallised from methyl alcohol as colourless crystals m.p.180-181°. It gave no yellow colour with tetranitromethane.

Mixed melting point with authentic sample of diacetyl bromolactone of echinocystic acid was undepressed.

Analysis. Found: C 63.4% H 7.9%

Calc.for $C_{34}H_{51}O_6Br$ C 64.2% H 8.1%

I.R. Spectra: $\lambda^{nujol}_{max.}$ 5.65; 5.83; 8.03 μ

Isolation of sugars:

The hydrolysate obtained by the hydrolysis of the saponin with sulphuric acid was neutralised with freshly precipitated barium carbonate and filtered. The precipitate of the barium sulphate was washed with hot water to remove any sticking sugars. The neutralised sugar solution and the washings were combined and evaporated to dryness in a vacuum oven at 35-40°. This gave a light brown syrup.

The hydrolysate was also neutralised by passing through a column of Amberlite IRA 400 ion exchange resin and the neutralised solution was evaporated to dryness in vacuum oven at 35-40°.

Paper chromatography of sugars:

The syrupy residue of the sugars was dissolved in a few drops of water and chromatographed on Whatman filter paper No.1 along side with authentic sugars using butanol:ethanol: water (40:11:19) as solvent mixture and utilising descending technique. The spots were revealed by spraying with p-anisidine phosphate[214] or aniline hydrogen phthalate[215] solutions. It showed the presence of the four sugars, glucose, arabinose, xylose and rhamnose. The absence of ketohexoses was found out by spraying the chromatogram with urea-HCl reagent[216] when no spots were seen. The Amberlite I.R.A.400 neutralised solution also showed the presence of the same four sugars.

5. Study of the seeds of Albizzia procera Benth. from Maharashtra.

Defatting: The finely powdered seeds (500 gms) obtained from the Silviculturist Maharashtra were extracted with light petroleum ether in a soxhlet apparatus. The recovery of the solvent gave 30 gms. of a greenish yellow oil.

Isolation of saponin: The defatted seed powder was exhausted in a soxhlet apparatus. The alcohol was recovered under reduced pressure. An oily residue was obtained, which was treated successively with petroleum ether, ether, chloroform, carbon-tetrachloride and acetone. It was then

dissolved in alcohol. filtered, and then added dropwise to a large volume of ether. An insoluble precipitate was obtained. which was redissolved in alcohol and reprecipitated in a similar way. The operation was repeated 3 times, and finally the precipitation was done in a large volume of acetone. The precipitate was filtered, washed with dry acetone and dried in a vacuum desiccator over calcium chloride. A light cream coloured hygroscopic powder (80 gms) was obtained which gave all the tests for saponin such as colour reactions, abundant foams on shaking with water toxicity to fishes in low concentrations etc.

Hydrolysis of the saponin.

The saponin (1 gm) was dissolved in water (400 cc) and hydrolysed with sulphuric acid (5%) by heating first on a boiling water bath for two hours and thereafter completing the hydrolysis by refluxing for another hour. A precipitate began to appear in about 20 minutes time, which went on increasing till the hydrolysis had completed. It was cooled and filtered. The precipitate was washed with water, free of sulphuric acid and dried in the air.

Purification of the genin.

The crude genin (3 gm) was refluxed with alcoholic potassium hydroxide (150 cc; 6%) for half an hour and thereafter the volume of alcohol was reduced to one-third

by distillation under reduced pressure. The solution was diluted with 500 cc of water and left overnight. No solid product separated out. It was then extracted four times with ether in order to separate any neutral genin present. The ether was washed with water free of alkali and evaporated to dryness. No neutral genin could be obtained.

The alkaline solution left after ether extraction was heated on a boiling water bath to remove traces of the ether present in the solution. It was then acidified with hydrochloric acid. A precipitate was obtained, which was filtered, washed with a large quantity of water and dried.

Acetylation.

The acid genin (500 mg) was dissolved in pyridine (20 cc) and acetic anhydride (15 cc) was added to it. After 24 hours, the contents were poured drop by drop and with continuous stiring into water containing ice pieces. A slightly brownish precipitate was formed, which was filtered, washed with water free of pyridine and acetic acid and dried. It crystallised from methyl alcohol in colourless fine needles m.p. 288-90°. It depressed the melting point when mixed melted with machaerinic acid acetate and gave a yellow colour with tetranitromethane. The same results were obtained by acetylation with hot

pyridine method and acetic anhydride and sodium acetate method. The genin was acetylated at room temperature with acetic anhydride and pyridine employing different time intervals(6 hrs., 4 hrs., and one hour). The same product m.p.288-90° was obtained in all cases.

Analysis. Found: C 77.08% H 9.36%

Calc.for $C_{32}H_{48}O_4$ C 77.37% H 9.74%

I.R. Spectra: $\lambda^{\text{nujol}}_{\text{max.}}$ 5.69 8.05 μ

Deacetylation of the acetate.

The acetate m.p.288-90° (500 mg) was refluxed with alcoholic potassium hydroxide (60 cc; 5%) for two hours. It was diluted with water and acidified with hydrochloric acid. A colourless precipitate was obtained, which was filtered and washed with water, till the washings became neutral. It was crystallised from methyl alcohol in colourless crystals, m.p.268-72°. It gave a positive test with tetranitromethane. It did not depress the melting point when mixed melted with machaerinic acid. 184
The mixed melting point was 268-70°.

I.R. Spectra: $\lambda^{\text{nujol}}_{\text{max.}}$ 2.85, 5.9 μ

Methyl ester.

The genin (500 mg) was dissolved in ether (200 cc) and an ethereal solution of diazomethane was added to it

in excess. The contents were left overnight at room temperature. The excess of diazomethane along with the ether was then removed by heating on a water bath. A residue was obtained, which was crystallised from methyl alcohol in fine needles m.p.224-27°. Mixed m.p. with methyl machaerinate was 225-22°. (Cf. Methyl machaerinate m.p.224-5)

Analysis. Found: C 75.7% H 10.4%

Calc.for $C_{31}H_{50}O_4$ C 76.49% H 10.36%

I.R. Spectra: $\lambda^{nujol}_{max.}$ 2.88 , 5.82 μ

Acetyl methyl ester from the acetate m.p.288-90°.

To a solution of the acetate (300 mg) in ether (200 cc) was added an excess of the ethereal solution of diazomethane and the flask was left for 24 hour. Excess of diazomethane and the ether was evaporated by heating on a water bath. A colourless product was obtained which was crystallised from methyl alcohol in fine needles m.p.288-90°. Mixed melting point with diacetyl methyl machaerinate was 256-58° (Cf.diacetyl methyl machaerinate m.p.278-80°)[184].

It did not depress the melting point, when mixed melted with the starting material.

Acetyl methyl ester from the methyl ester m.p.224-27°.

The methyl ester (300 mg) was acetylated with acetic anhydride (15 cc) in pyridine solution (20 cc) and

left at room temperature for 24 hours. The contents were then poured dropwise in a large quantity of ice cold water when a precipitate was formed. It was filtered, washed with water and dried. Colourless needles m.p. 281-82° were obtained on crystallisation from methyl alcohol. Mixed melting point with diacetyl methyl machaerinate was 282-83° (Cf. diacetyl methyl machaerinate m.p. 278-80°).[184]

Analysis. Found: C 74.3% H 8.6%

Calc.for $C_{35}H_{54}O_6$ C 73.64% H 9.53%

I.R. Spectra: $\lambda_{max.}^{nujol}$ 5.82, 8.1 μ

6. Study of the flowers of Albizzia lebbek,Benth.

Defatting and isolation of the saponin:

The flowers of albizzia lebbek,Benth (200 gm) were well extracted with light petroleum ether (40-60°) and then exhausted with 95% alcohol in a soxhlet apparatus. The solvent was recovered under reduced pressure, when a brown syrupy mass was obtained. It was dissolved in alcohol and filtered. The alcohol was recovered, when a gummy residue was obtained. This was extracted with ether, petroleum ether, carbon tetrachloride chloroform and acetone successively. An insoluble mass left over was dissolved in a small quantity of alcohol and precipitated by addition to a large volume of ether. The

precipitate was again dissolved in alcohol and precipitated with ether. The process was repeated three times. Finally the isolate was decolourised with activated animal charcoal and precipitated with acetone. It gave a colourless hygroscopic powder (10 gms) which gave all the tests for saponins.

Hydrolysis of the saponin:

The saponin (1 gm) was dissolved in water (1 litre) and hydrolysed with sulphuric acid (7%) by heating the solution first on a boiling water bath for an hour and thereafter completing the hydrolysis by boiling the solution for another hour. After about 20-30 minutes of heating, a precipitate began to appear which went on increasing till the completion of the hydrolysis. It was filtered, washed with water free of acid and dried in an air oven. The dried genin was dissolved in alcohol and decolourised with activated charcoal.

Separation of the Acid and neutral genin:

The genin (1 gm) was refluxed with methyl alcoholic potassium hydroxide (100 cc: 5%) for one hour and thereafter, half of the solvent was distilled off. The solution was then diluted with water (600 cc) and extracted thrice with ether. The ethereal extracts were combined and washed free of alkali. Recovery of the ether left no neutral genin. The alkaline solution, after its ether extraction was acidified with hydrochloric acid, which precipitated the acid

sapogenin. It was filtered , washed several times with water and then dried at room temperature.

Acetylation of the acid genin.

The genin (200 mg) was dissolved in pyridine (20 cc) and heated with acetic anhydride (15 cc) on a boiling water bath for one and a half hour. The contents were cooled to room temperature and poured dropwise into ice-cold water. The precipitate obtained was filtered and washed several times with water to remove pyridine. It was crystallised from methyl alcohol in colourless needles m.p.257-62°. It did not depress the melting point, when mixed melted with an authentic sample of echinocystic acid (Cf.diacetyl echinocystic acid m.p.272-75°, 248-49°)[213,183].

Analysis. Found: C 73.1% H 8.7%

Calc.for $C_{34}H_{52}O_6$ C 73.35% H 9.41%

I.R. Spectra: $\lambda_{max.}^{nujol}$ 5.81, 5.90, 8.09 μ

Deacetylation of the acetate:

The acetate (100 mg) was refluxed with methyl alcoholic potassium hydroxide (50 cc; 10%) for three hours on a water bath. The mixture was cooled to room temperature and poured in a large amount of water. It was acidified with hydrochloric acid, when the acid genin was obtained, which was filtered and washed free of the acid.

The genin was crystallised from isopropyl alcohol as colourless crystals m.p.297-301°. No depression was noted when mixed melted with echinocystic acid (Cf.echinocystic acid m.p.305-312°; 291-293°)[213,183].

I.R. Spectra: $\lambda_{max.}^{nujol}$ 2.87, 5.97 μ

(vi) Acetyl Methyl ester.

To a solution of the acetate (100 mg) in ether (300 cc) was added an excess of the ethereal solution of diazomethane. The mixture was left for 16 hours. The ether and the excess of diazomethane was then evaporated on a water bath. A solid residue was obtained, which was crystallised from methyl alcohol as colourless needles m.p.201-202°. It did not depress the melting point when mixed melted with diacetyl methyl echinocystate (Cf.echinocystic acid diacetyl methyl ester m.p.200-201°)[213,183].

I.R. Spectra: $\lambda_{max.}^{nujol}$ 5.75, 8.09 μ

Isolation of anthoxanthin glycoside.

The alcoholic extract of the flowers left a residue on evaporation of the alcohol. It was dissolved in water and the aqueous solution extracted six times, with

n-butyl alcohol when the last extraction was colourless. The butyl alcohol fractions were combined and recovered under reduced pressure. More butyl alcohol was added and recovered in order to remove water completely. The residue of the saponin and the glycoside thus obtained was dissolved in alcohol, filtered and then added dropwise to a large volume of acetone, which precipitated the saponin. The saponin was filtered off and the acetone solution was evaporated to dryness. A gummy residue obtained was again dissolved in alcohol and reprecipitated with acetone. The operation was repeated three times which removed all the saponin. The acetone solution after removal of the insoluble saponin fraction, on evaporation yielded a product which gave a salmon pink colouration with magnesium and hydrochloric acid [illegible]. All attempts at crystallisation were fruitless.

Hydrolysis of the glycoside:

The glycoside (200 mg) was dissolved in water (400 cc) and hydrolysed with sulphuric acid (10%) on a boiling water bath for three hours. A solid product separated out, which was filtered and washed with water free of the acid.

Purification of the aglycone:

To a dilute alcoholic solution of the aglycone, were added two drops of dilute lead acetate solution. The precipitated impurities were filtered off. Hydrogen sulphide was passed into the filtrate to remove any lead acetate in solution. The solution was filtered and evaporated to dryness under reduced pressure on a water bath at 50°C. This yielded a product which gave acidic cyanidin and Wilson's boric acid tests [201].

Chromatography of the aglycone.

A small quantity of the aglycone dissolved in a few drops of alcohol was deposited on Whatman filter paper no.1. It was chromatographed alongside with an authentic sample of quercetin (L.Light & Co. England) using acetic acid:Water (60:40) as solvent mixture [202] and utilising ascending technique. The chromatogram was run for 16 hours. dried and sprayed with sodium carbonate solution or ferric chloride solution [203]. It showed three clear spots. The examination under ultra-violet light or ultra-violet light and ammonia vapours also revealed the same three spots. One of the spots was found to be identical with quercetin.

7 - The seeds of Luffa aegyptica Mill.

Defatting. The well powdered seeds (1 kg) of Luffa aegyptica Mill were exhausted with light petroleum ether (40-60°) in a soxhlet apparatus. The recovery of the solvent gave a deep green oil (104 gm) i.e. 10.4% yield.

Isolation of sapcnin:

The well defatted seed powder (800 gm) was exhausted with ethanol. The recovery of the solvent left a white amorphous powder, which was filtered on a buckner funnel and washed with ether and acetone. This gave a white colourless powdery substance.

Isolation of the sapogenin:

The sapcnin (5 gm) was dissolved in large amount of water (2 litres) and hydrolysed with sulphuric acid (10%) by heating first on a water bath for one hour and then refluxing for another hour. The sapogenin which separated as colourless precipitate was filtered and washed with water till the washings were neutral.

Separation of acid and neutral sapogenins.

The crude sapogenin (5 gm) was dissolved in ether (1 litre) and extracted with aqueous caustic soda solution (4%; 100 cc). The operation was repeated five times. The ethereal layer was washed with water, free of alkali. The ether on evaporation gave a colourless neutral resin.

Acetylation of Acid sapogenin.

The acid sapogenin (500 mg) was refluxed with fused sodium acetate (2 gm) and acetic anhydride (30 cc) for four hours. It was then poured in a mixture of ice and water and left over night. The precipitate was filtered, washed free of acid and crystallised from methanol, when two types of crystals were obtained which were separated into two different products by fractional crystallisation, m.p.266-68° and m.p.176-81°. Both the acetates gave a yellow colour with tetranitromethane.

Anal. Found: C 77.6% H 9.8%
Calc. for $C_{32}H_{50}O_6$: C 77.1% H 10.1%

I.R. Spectra: ν_{max}^{nujol} 5.82, 5.98, 8.04 μ

Deacetylation of acetate m.p.266-68°.

The acetate m.p.266-68° (300 mgm) was refluxed with methyl alcoholic sodium hydroxide solution 10% (30 cc) for one hour. The solution was cooled and diluted with water and the sodium salt was decomposed by the addition of an excess of hydrochloric acid. The precipitate formed was left over night and filtered, washed free of acid and crystallised from methyl alcohol as colourless needles m.p.302-304° (Cf. oleanolic acid m.p.310°), mixed melting point with oleanolic acid 302-303°. It gave positive Liebermann-Burchard reaction and yellow colour with tetranitromethane.

Methyl ester:

The genin (200 mgm) m.p.302-304° was dissolved in ether and treated with an excess of an ethereal solution of diazomethane. It was left over night and thereafter excess of diazomethane and ether was removed on a water bath by boiling. The product was crystallised from methanol as colourless needles m.p.198-99° (Cf.Methyl oleanolate m.p.198-200°) mixed m.p. with methyl oleanolate was 199-200°). It gave positive reaction for C = C double bond with tetranitromethane.

Acetyl methyl ester.

The acetate (200 mg) m.p.266-68° was dissolved in ether (200 cc) and methylated by the addition of an excess of diazomethane for 24 hours. The ether was removed by heating on a water bath and the product obtained was crystallised from methyl alcohol containing a little chloroform. It gave colourless plates m.p.220-21°. Mixed m.p. with an authentic sample of acetyl methyl oleanolate was 220-21°. It gave positive reaction with tetranitromethane.

I.R. Spectra: $\lambda_{max.}^{CHCl_3}$ = 5.8,8.0 μ

Found. C 77.6% H 9.7%

Calc.for $C_{33}H_{52}O_4$ C 77.28% H 10.22%

Acetylation of the neutral genin:

The neutral genin (200 mg) was dissolved in pyridine (20 cc) and acetic anhydride (15 cc) was added to it. The contents were left for 18 hours at room temperature and then poured in ice-cold water, drop by drop. It gave a precipitate which was left overnight, then filtered and washed free of pyridine. On crystallisation from methyl alcohol it gave colourless needles m.p. 262-64°. It gave no colour with tetranitromethane.

Wolff-Kishner reduction of the Neutral genin Acetate.

The neutral genin acetate (100 mg) was dissolved in digol (30 cc), containing sodium (1 gm), and refluxed with hydrazine hydrate (5 cc) for four hours at 180°. The temperature was then raised to 210° and refluxing was continued for 12 hours. The contents were cooled and diluted with water (200 cc). It was extracted with ether. The ether on recovery gave no product. The ether extracted solution was acidified with hydrochloric acid, and reextracted with ether. The ether was washed with water free of acid, and evaporated to dryness. A solid product obtained was crystallised from methyl alcohol in fine needles m.p. 288-92°. The mixed melting point with oleanolic acid was 290-92°. (Cf. Oleanolic acid, m.p. 310°). It gave a yellow colour with tetranitromethane.

Acetylation of the Wolff-Kishner reduction product:

The substance (60 mg) was acetylated with acetic anhydride and pyridine in the usual manner. The acetate crystallised from methyl alcohol in fine needles m.p. 264-66°. Mixed m.p. with oleanolic acid acetate was 263-65°.

Wolff-Kishner reduction of the acetate m.p. 176-81°.

The acetate (60 mg) m.p. 176-81° was refluxed with diçol 20 cc, sodium (800 mg) and hydrazine hydrate (4 cc) at 180° for 4 hours, and then refluxed at a temperature of 210° for 12 hours more. It was cooled, diluted with water and extracted several times with ether. The ether on recovery gave no product. The alkaline solution on acidification and extraction with ether gave a product, which was crystallised from methyl alcohol in fine needles m.p. 288-92°. Mixed melting point with oleanolic acid was not depressed.

Acetylation of the reduction product, m.p. 288-92°

The substance (50 mg) was acetylated with acetic anhydride and pyridine (2cc; 5cc) in the cold for 24 hours. It was poured in water, filtered, washed with water and crystallised from methanol in fine needles, m.p. 263-66. It did not depress the m.p., when mixed melted with oleanolic acid acetate.

CONCLUSIONS

C O N C L U S I O N S

From the present study which extends to the members of only two families, the following conclusions can be drawn.

A. Family Leguminosae.

1). The seeds of Sesbania aegyptica,Pers. from Uttar Pradesh yield a mixture of saponins which on hydrolysis gives oleanolic acid (A_1) and a neutral sapogenin.

2). The seeds of Sesbania aculeata, Pers. from Uttar Pradesh also yield oleanolic acid (A_1) and another neutral sapogenin, different from the one obtained from Sesbania aegyptica. Both are present in the seeds as saponins.

3). The seeds of Albizzia odoratissima,Benth from the state of Maharashtra contain a mixture of saponins, which on hydrolysis yields two acid sapogenins,the acetates of which had m.p.260-65 and m.p.228-34 respectively. They have been identified as machaerinic acid (A_2) and acacic acid (A_3).

4). The seeds of Albizzia odoratissima,Benth from Uttar Pradesh are different in chemical constituents from those of Maharashtrian origin (loc.cit.) and yield a new saponin m.p.227-28 which has been named Odoratissimin. The hydrolysis of odoratissimin, yields echinocystic acid (A_4) as sapogenin and glucose, arabinose, xylose and rhamnose as sugar moieties.

5). The seeds of Albizzia procera,Benth from the state of Maharashtra, yield a new saponin which has been named Maharrocin. It is quite different in the constitution from

the proceranin, a tetraglycoside of Machaerinic acid (earlier reported by Warooq, Varshney and Hasan, Arch.der Pharm.,1959, 292,57; Compt.rend.,1958,246,3251; Current Sci.,1958,27,489, from the seeds of Albizzia procera from Madhya Pradesh). The Mahaprocin on hydrolysis yields a new acid sapogenin m.p.268-72, acetyl lactone m.p.288-90, methyl ester m.p.224-27 and acetyl methyl ester m.p.231-32 and has been named as Proceric acid (A_5). The proceric acid is 3,21-dihydroxy-Δ^{12}-oleanene-28-oic acid having 21-hydroxyl and 28-carboxyl groups in the same plane, making the lactonisation between them very easy.

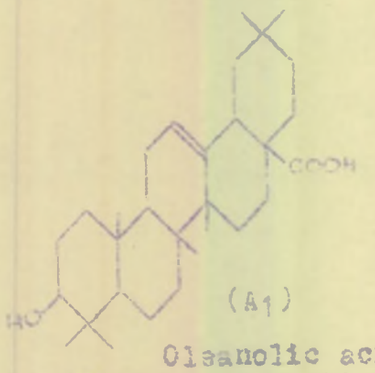
6). The flowers of Albizzia lebbek,Benth from Uttar Pradesh yield a saponin and a mixture of anthoxanthin glycosides. The saponin on hydrolysis yields an acid sapogenin identified as echinocystic acid (A_4). Out of the three components, obtained from the hydrolysis product of the above anthoxanthin glycosides, only one has been identified as quercetin.

B. Family Cucurbitaceae.

7). The seeds of Luffa aegyptica,Mill (Black Variety) yield a mixture of three sapogenins, two acid and a neutral one all occuring as saponins. One of the acid sapogenins has been identified as oleanolic acid (B_1). The second acid sapogenin has been identified as gypsogenin (B_2) and the neutral one, as acetyl gypsogenin lactone (B_3). Actually the last one is the lactonised form of the second (B_2) acid genin.

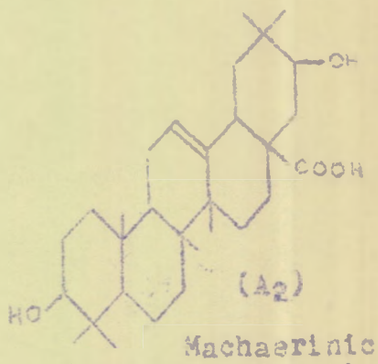
The following are the chemical structures of all the triterpenes which have been encountered in the members of the family Leguminosae, and Cucurbitaceae.

A- Family Leguminosae.



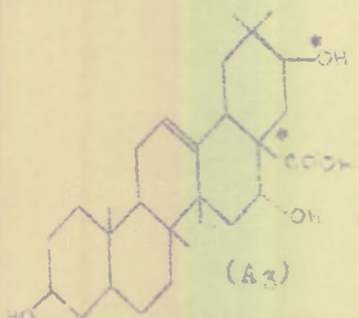
(A_1)

Oleanolic acid
(Sesbania aegyptica & S. aculeata).



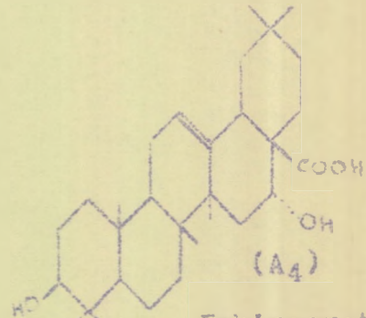
(A_2)

Machaerinic acid
(Albizzia odoratissima from Maharashtra).



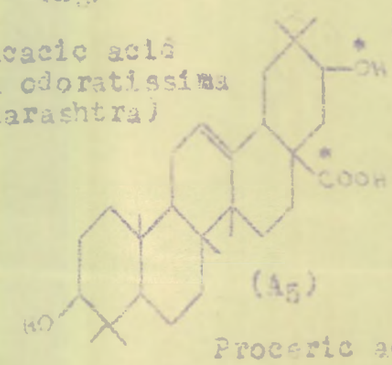
(A_3)

Acacic acid
(Albizzia odoratissima from Maharashtra)



(A_4)

Echinocystic acid
(Albizzia odoratissima from Uttar Pradesh)



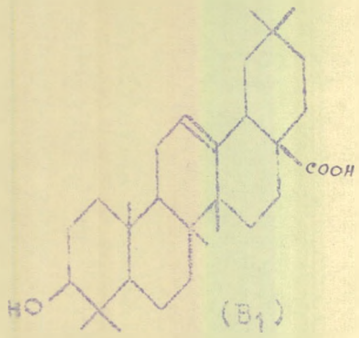
(A_5)

Proceric acid
(Albizzia procera from Maharashtra)

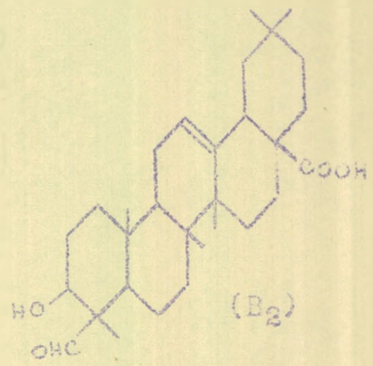
*Same plane making very easy lactonisation possible.

B - Family Cucurbitaceae

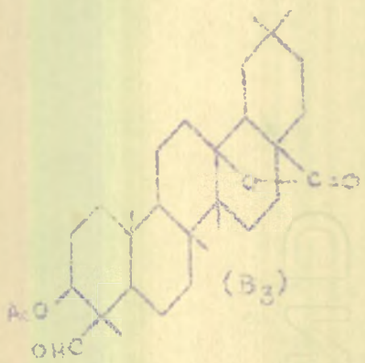
(Luffa aegyptica Mill. Black variety).



Oleanolic acid



Gypsogenin



Acetyl gypsogenin lactone.

The most interesting fact that emerges from the study of the family Leguminosae is that all these compounds are the members of the β-amyrin series. Leaving aside the ubiquitous 3β-hydroxyl group, the hydroxylation has taken place in all these products isolated (from A_1 to A_5) in two positions i.e. 16 and 21 which fall in only two rings, D and E. A unique feature found in two of the members (A_3 and A_5) is that the orientation of the carboxyl group and one of the

hydroxyl group is the same making the lactonisation between them not only easy but extremely easy, which has not been encountered so far in any acids from any other family. Further there is another striking fact that all these genins occur as saponins and none has been noted in the free state, in this family. It appears that there exists a very close biogenetic relationship between these acids. But to ascertain the existance of any such relationship, it will be necessary to make a thorough study of other members of this family.

It is also to be noted that the locality from where the seeds are obtained, plays an important part on the nature of the constituents, chiefly on the oxygenation. The seeds obtained from two different localities are found to be different in the nature of sapogenins, differing in the position of oxygen attachment or in the orientation of the functional groups. Therefore, a new system of classification of the plants on the basis of their chemical constituents, also deserves consideration.

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Saponins and Sapogenins IV*

Isolation of Oleanolic Acid from *Sesbania Aegyptica* Pers.

By M. O. FAROOQ, I. P. VARSHNEY, and MOHD. S. Y. KHAN

The seeds of *Sesbania Aegyptica* Pers. have been found to contain two triterpenic sapogenins, one an acid sapogenin and the other a neutral one. The acid genin has been identified as oleanolic acid by a direct comparison of the melting point and the infrared spectra of the methyl ester of the acetate with an authentic sample.

Sesbania Aegyptica Pers., locally known as "Ravasin," is a member of the family *Leguminosae*, sub-family *Papilionatae*, and grows wild throughout the plains of India. Owing to its rapid growth it is being increasingly used as a hedge plant and it is a good cattle feed. The seeds are used in cases of the enlargement of the spleen (1). A number of the plants of the family *Leguminosae* have been found to contain saponins and sapogenins (2–5), but the only record about this plant is that it contains no saponin (6). During our work on the fat content (7) of the seeds of the plant, the presence of a saponin was indicated and therefore its systematic examination for the saponin content was taken up.

The seeds of *Sesbania Aegyptica* Pers. were procured from M/S. N. Cooper & Co., Poona, as well as obtained from the plants grown on the University campus. A quantity of the finely powdered, defatted seeds were extracted with ethanol. The recovery of the solvent left an oily residue which was successively treated with ether, petroleum ether, carbon tetrachloride, and acetone. The residue thereafter was taken up in alcohol and precipitated by addition to a large amount of ether. This precipitation was repeated a number of times. This gave colorless saponin which became a brown syrup on exposure to atmosphere and satisfied all the tests for saponin. The saponin was dissolved in a large amount of water and hydrolyzed with sulfuric acid. The genin obtained was filtered and washed free of the acid. The failure of a number of attempts at crystallization from different solvents suggested the genin to be a mixture and, therefore, it was refluxed with a solution of alcoholic potassium hydroxide and extracted with ether. Evaporation of the ethereal layer gave a neutral genin while the alkaline solution, on treatment with an excess of hydrochloric acid, gave a precipitate of an acid genin. This acid genin gave an acetate which, on crystallization from methanol, had m. p. 261–263°, $[\alpha]_D$ = +72.7. The acetate gave positive response to all the usual tests for a triterpene.

The resemblance of the infrared spectrum of the acetate with the spectra of the acetates of the triterpenic acids of β-amyrin group (methylene bending absorption) was very close and did not resemble at all the infrared spectra of the acetate of the steroidal sapogenins (finger print region) (2, 4).

The deacetylation of the acetate gave an acid genin, m. p. 285–288°, $[\alpha]_D$ = +80. The analysis of the acetate indicated the presence of one hydroxyl and one carboxyl group in a pentacyclic triterpenic compound.

The carboxyl function which was readily detectable in the infrared spectra was fixed by the formation of a methyl ester, m. p. 195–198°, $[\alpha]_D$ = +76. The fact that it was obtained with diazomethane and not with methanolic hydrochloric acid, coupled with the difficulty of its hydrolysis, suggested the attachment of the carboxyl group to a tertiary carbon atom (i. e., to C-17). The methylation of the acetate with diazomethane gave an acetyl methyl ester, m. p. 217–219°, $[\alpha]_D$ = +72.7. The genin and all its derivatives showed unsaturation with tetranitromethane.

The genin on oxidation with chromic acid gave a ketone which gave a positive Zimmermann test (8) showing the position of the carbonyl group at C-3. The ketone gave a 2,4-dinitrophenylhydrazone without difficulty.

The relation with the β-amyrin group was further established by the examination of the U. V. spectrum of the product of oxidation of the acetate with selenium dioxide. The product obtained on oxidation could not be crystallized and showed the characteristic triple ultraviolet absorption maxima ($\lambda_{max.}^{EtOH}$ 241, 249, 255 mμ) of a diene.

By analogy with all the acids of the β-amyrin group the position of one of the hydroxyl groups has been assumed to be at C-3 in ring A, and that of the carboxyl group at C-28 attached to C-17. The comparison of the physical constants of the

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For earlier three parts see (5).

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genin and its derivatives with all the known acids of the β-amyrin group carrying one hydroxyl group indicates the present genin to be identical with oleanolic acid (Table I).

The comparison of the infrared spectra (Fig. 1) and mixed melting point of the acetyl methyl ester of the genin with authentic samples of acetyl methyl oleanolate obtained from *Albizzia lebbek* (2) and *Randia dumetorum* (9) confirmed the identity of the genin as oleanolic acid.

TABLE I.

	Oleanolic Acid m. p.	Oleanolic Acid $[\alpha]_D$	Present Genin m. p.	Present Genin $[\alpha]_D$
Genin	310	+80	285–288	+80
Acetyl oleanolic acid	268	+74.5	261–263	+72.7
Methyl oleanolate	198–200	+75	195–198	+76
Acetyl methyl oleanolate	219–220	+70	217–219	+72.7

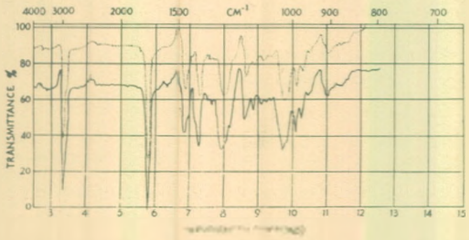

Fig. 1.—Comparison of infrared spectra. Solid line shows present genin acetyl methyl ester. Broken line shows acetyl methyl oleanolate.

EXPERIMENTAL

All the melting points recorded in this paper have been taken on a Kofler hot microscopical stage and are corrected. The infrared spectra have been taken in chloroform solution at the Presidency College, Madras, using a double beam Perkin-Elmer spectrometer Model 137 (Infracord) and interpreted by one of the authors (I. P. V.). The microanalyses recorded here have been done at the Department of Chemical Technology, University of Bombay. The ultraviolet spectra have been measured using a Beckman spectrophotometer Model DU.

Defatting.—Well-powdered seeds (1 Kg.) were exhausted in a Soxhlet extractor with light petroleum ether (40–60°). The recovery of the solvent left a greenish oil (55 Gm.). The exhausted seeds were dried before further operation.

Extraction.—The defatted seed powder was exhausted with 95% alcohol in a Soxhlet extractor and the solvent recovered leaving a brown syrupy liquid. The residual syrup was dissolved in ethanol and filtered. The alcohol was recovered and the solid residue extracted with ether, petroleum ether, carbon tetrachloride, and acetone, successively. This gave a brown syrupy mass, which was dissolved in a little alcohol and added to a large amount of ether, which precipitated the saponin. This process of dissolution in alcohol and precipitation by ether was repeated three times. It gave colorless saponin which turned to a syrup on exposure to air, and gave all the tests for saponin.

Isolation of Sapogenin.—The brown syrupy mass (25 Gm.) was dissolved in water (6 L.) and hydrolyzed with sulfuric acid (300 Gm.) by heating the solution first on a boiling water bath for an hour and thereafter completing the hydrolysis by boiling the solution for another hour. After about twenty to thirty minutes a precipitate began to appear which went on increasing until the hydrolysis was completed. It was then filtered and washed with water until the filtrate was neutral. It was dried in an air oven at 80°. The dried genin was dissolved in alcohol and decolorized with charcoal. All attempts at crystallization from various solvents proved fruitless.

Separation of Acid and Neutral Genin.—The crude genin (1 Gm.) was heated with alcoholic caustic potash (20 Gm. KOH in 300 cc. of alcohol) for a half-hour and then half the solvent was distilled off. The solution was then diluted with water (2 L.) and extracted three times with ether. The ethereal extracts were combined and washed free of the alkali. It was dried over sodium sulfate (anhydrous) and removal of the ether left a neutral substance in the flask.

The alkaline solution was acidified with hydrochloric acid when it gave a precipitate. This was filtered, washed free of the acid, and dried.

Acetylation.—The acid genin obtained as above was acetylated by treatment with pyridine and acetic anhydride in the cold for eighteen hours. It was poured into ice water and filtered. The precipitate was washed free of the acid and pyridine and crystallized twice from methanol to obtain the product in fine, colorless needles, m. p. 261–263°, $[\alpha]_D^{25} = +72.7$ (C = 0.165 $CHCl_3$), yield = 500 mg. It gave a positive test with tetranitromethane, $\lambda_{max.}^{CHCl_3}$ 5.8, 5.9, and 8.0μ

Anal.—Calcd. for $C_{32}H_{50}O_4$: C, 77.11; H, 10.40. Found: C, 77.0; H, 9.8.

Deacetylation.—The acetate (250 mg.) was refluxed for two hours with 15 cc. of 5% methanolic potassium hydroxide. The solution was diluted with 200 cc. water and left overnight at room temperature. It did not yield any crystalline potassium salt. The solution was acidified with hydrochloric acid and the precipitate formed was washed free of the acid and crystallized from methanol, m. p. 285–288°, $[\alpha]_D^{26} = +80$ (C = 0.100 $CHCl_3$). It gave a positive test with tetranitromethane.

Selenium Dioxide Oxidation.—Acetate (100 mg.) in 15 cc. acetic acid was heated under reflux with 100 mg. freshly sublimed selenium dioxide for two hours. It was poured into water and extracted with ether. It could not be crystallized. $\lambda_{max.}^{EtOH}$ = 241, 249, 255 mμ.

Methylation.—The acid genin (200 mg.) was dissolved in 200 cc. ether and an excess of ethereal solution of diazomethane added. It was left overnight, the excess of diazomethane was removed on a water bath, and the product was crystallized from methanol, m. p. 195–198°, $[\alpha]_D^{26} = +76$ (C = 0.098 $CHCl_3$). The substance gave a positive test with tetranitromethane.

Anal.—Calcd. for $C_{31}H_{50}O_3$: C, 79.1; H, 10.6. Found: C, 78.2; H, 10.8.

Demethylation.—The methyl ester (100 mg.) was refluxed with 100 cc. of 5% methanolic caustic potash for half an hour. The starting material (80%) was recovered unchanged and only 20% of the ester was demethylated. The genin obtained was confirmed by melting and mixed melting points.

Acetyl Methyl Ester.—The acetate (100 mg.) was dissolved in ether and an excess of a solution of a diazomethane added to it. After a contact of twenty hours ether was removed and the residue crystallized from methanol, m. p. 217–219°, $[\alpha]_D^{26} = +72.7$ (C = 0.165 $CHCl_3$). It gave a yellow color with tetranitromethane. $\lambda_{max.}^{CHCl_3}$ = 5.8, 8.0 μ.

Anal.—Calcd. for $C_{33}H_{52}O_4$: C, 77.29; H, 10.22. Found: C, 77.3; H, 9.7.

Oxidation of the Genin.—A solution of chromic acid (100 mg.) in 15 cc. of 80% acetic acid was added to 100 mg. of the genin dissolved in 20 cc. acetic acid. After leaving it for half an hour at room temperature, 100 cc. alcohol was added to destroy the excess chromic acid. The solution was left for fifteen minutes and then the alcohol was distilled off under reduced pressure. The residue was dissolved in aqueous alcohol and extracted with ether. The ethereal solution was washed well with water and evaporated to dryness. The product crystallized from methanol as fine needles, m. p. 156–160°. It gave a positive Zimmermann test.

2,4-Dinitrophenylhydrazone.—The ketone (50 mg.) was dissolved in 10 cc. alcohol and heated on a water bath. It was then mixed with a solution of 50 mg. 2,4-dinitrophenylhydrazine in 10 cc. alcohol and 0.4 cc. hydrochloric acid. It was left at room temperature for five minutes when a precipitate was formed. It was kept overnight, filtered, washed, and recrystallized from ethanol, m. p. 251–255°.

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